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PHYSIOLOGICAL ZOÖLOGY

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THE DETERMINATION OF THE REGENERATING HYDRANTH IN TUBULARIA

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PREVIOUS work (Miller, 1937; Barth, 1938*a*) shows that the concentration of oxygen is an important factor both in the rate of regeneration and in the determination of the polarity of *Tubularia* stems. More recently, however, Miller (1939, 1942,)Rose and Rose (1941), and Goldin (1942*a*) have shown that certain inhibitors produced by the stem also play a role during regeneration. These two groups of observations suggest that a ratio involving the concentration of oxygen over the concentration of inhibitors, $[O_2]/[\Sigma I]$, determines the rate of regeneration and the polarity of the stem (Goldin, 1942*a*, *b;* Barth, 1944). Thus, either decreasing the concentration of oxygen by use of gas mixtures (Barth, 1940*a*) or increasing the concentration of inhibitors by allowing them to accumulate in the sea water (Rose and Rose, 1941; Miller, 1942) has the effect of decreasing the rate of regeneration, often resulting in complete inhibition of regeneration or reversal of polarity when one end of the stem is treated.

It is the purpose of these investigations (1) to work out the relation between O_2 concentration, rate of O_2 consumption of the stems, and rate of regeneration; (2) to study the antagonism between high concentrations of oxygen and inhibitors; and (3) to determine the periods during regeneration when oxygen and inhibitors act.

I. GENERAL METHODS

The stems of *Tubularia* were carefully selected for uniform length, diameter, and appearance of stem. Stems which were damaged in collecting, which were crooked or had a large number of side branches, and which bore hydranths with large gonophores were discarded. After selecting all the stems necessary for an experiment the hydranths were cut off and discarded, and the stems were cut to the desired length. Random samples of these prepared stems were used for the various treatments involved in each experiment.

Regeneration was measured by (1) the length of the primordium of the hydranth just as it constricts from the stem and (2) the time, in hours, from the cutting of the stem to the formation of the primordium. Records of the diameter of stems were kept so that the volume of the primordium could be calculated. The diameter of the stem is fixed by the di-

ameter of the perisarc and is not subject to change by any treatment. Only the length of the primordium can be changed. In previous papers the rate of regeneration has been defined as the length of the primordium divided by time, so as to compare regeneration where these factors vary. Child (1941) and Miller (1942) have put forth objections to this definition; and their criticisms are, for the most part, correct. In this paper, therefore, the length of the primordium and the time for regeneration are considered as unrelated variables.

II. CONCENTRATION OF OXYGEN OXYGEN CONSUMPTION, AND REGENERATION

The concentrations of oxygen were obtained from the following mixtures of oxygen and nitrogen in cylinders: 100 per cent O_2; 50 per cent O_2 plus 50 per cent N_2; 10 per cent O_2 plus 90 per cent N_2; 5 per cent O_2 plus 95 per cent N_2; 2.5 per cent O_2 plus 97.5 per cent N_2. These gas mixtures were passed through Warburg manometer flasks containing the freshly cut stems of *Tubularia*. The O_2 consumption was determined in the usual way for about 24 hours, after which the stems were removed from the flasks, the lengths of the regenerating primordia were measured, and the times from cutting to the stage of the fully formed primordia were recorded. The stems were then dried and weighed on a microbalance for the calculation of the rate of O_2 consumption.

From Table 1 it is seen that, as the concentration of oxygen is decreased, (1) the oxygen consumption of the stem as a whole decreases, (2) the length of the primordium at the end of the stem decreases, and (3) the time for the formation of the primordium is increased.

The changes in O_2 consumption of the stems in various concentrations of oxygen are not caused by the changes in regeneration at the ends of the stems. This was pointed out in earlier preliminary experiments (Barth, 1940*a*) in which the O_2 consumption of regenerating stems was found to be no greater than the O_2 consumption of resting, nonregenerating stems. Thus, stems which were prevented from regenerating by means of ligatures at the ends consumed as much oxygen as stems which regenerated freely. This result was verified again in connection with the above experiments. The O_2 consumption of ligatured stems which showed no signs of regeneration for 32 hours consumed slightly more oxygen than a similar lot of stems which were not ligatured and which showed normal regeneration during the same 32-hour period. When the ligatures were removed from the ends of the stems, regeneration occurred, showing that the stems were not injured by those ligatures. Since regeneration does not increase the O_2 consumption of the stem as a whole, the change in O_2 consumption with change in O_2 concentration cannot be caused by a change in regeneration at the ends of the stem but rather by a change in the rate of oxidation along the entire length of the stem.

It would appear from the foregoing observation that, since the rate of O_2 consumption is high enough for regeneration in ligatured stems, the block or inhibition occurs at some other step in the process of regeneration. In other words, the rate of energy production is sufficient in ligatured stems, but the tissues cannot utilize this energy. Therefore, the block may be in the reactions which transfer the energy of oxidations to the endergonic (energy-absorbing) reactions (Kalckar, 1942) involved in regeneration. A similar case exists in the experiments of Moog and Spiegelman (1942), where regeneration is blocked, with little

change in O_2 consumption, by the use of phenylurethane. One interpretation of both kinds of inhibition of regeneration is that the utilization of energy is blocked.

However, there is a second possibility, namely, that such small amounts of the total energy from oxidation is necessary for regeneration that a change in O_2 consumption cannot be detected with the methods used. If, for example, such a value as 1 per cent of the O_2 consumption of the total be concerned with regeneration, the methods used to date would not detect this difference between regenerating and nonregenerating stems. In this connection it must be remembered that Child and Watanabe (1935) have shown an increased rate of reduction of methylene blue at the regenerating ends, as compared to the resting tissue, in *Corymorpha*. Whether this phenomenon is coupled with an increased O_2 consumption has not been demonstrated.

There is a third explanation for the lack of differences in the O_2 consumption of regenerating and nonregenerating stems. The over-all rate of O_2 consumption may not increase during regeneration, but the kind of substrates oxidized may change. Thus the nonregenerating stems may be oxidizing relatively more substrates which do not furnish energy for regeneration, while the regenerating stems oxidize relatively more of the substrates which do furnish energy for regeneration. The over-all O_2 consumption of nonregenerating and regenerating stems could thus be the same, but the substrates and the coupling of reactions could be different.

In any case, it is clear that changes in the process of regeneration have little or no effect on the over-all rate of O_2 consumption and could not bring about the observed differences in the over-all rate recorded in Table 1.

The interpretation suggested in this paper for the effect of changes in concentration of oxygen or the process of

TABLE 1

OXYGEN CONCENTRATION, OXYGEN CONSUMPTION, AND REGENERATION

O_2 = rate of O_2 consumption of stems in percentage of the rate in 100 per cent oxygen; L = average length of primordia in percentage of the average length in 100 per cent oxygen; t = average time for development of primordia in percentage of the average time in 100 per cent oxygen; temperature, 18.5° C.

Description		Oxygen Concentration (Per Cent)				
		100	50	10	5	2.5
No. 1: 125 stems 5 mm. long	O_2	100	93	82	78	55
	L	100	94	80	72	62
	t	100	100	97	110	137
No. 2: 125 stems 5–7 mm. long	O_2	100	113	85	71	53
	L	100	108	84	78	66
	t	100	102	102	107	124
No. 3: 100 stems 4–5 mm. long	O_2	100	100	118	96	63
	L	100	102	91	82	74
	t	100	98	102	117	127
No. 4: 150 stems 5–6 mm. long	O_2	100	.....	94	84	65
	L	100	.....	91	82	65
	t	100	.....	100	110	137

regeneration is: (1) The concentration of oxygen governs the rate of oxidations, and so the rate of energy production, of the stem. (2) The rate of energy production controls the size of the primordium and also the time necessary for regeneration. The increase in size of the primordium with a change from low to high O_2 concentrations brings up the whole question of the control of size. In a previous paper (Barth, 1938*b*) the suggestion was made that, because longer stems form larger primordia, the resting parts of the stem contributed substances to the regenerating ends. Similarly, the phenomenon of dominance was interpreted as the competition of the two ends for these substances. If these substances are energy-rich compounds built up at the expense of the oxidation of substrates in the cells of the entire stem, then with increase in O_2 concentration the entire stem would form larger amounts of these energy-rich substances. Thus, more of the tissue at the ends of the stem would have the energy necessary for regeneration, and the primordium would be larger. The increase in bipolar forms with increase in O_2 concentration (Barth, 1938*a*) could be explained in the same way.

At this point the question arises: Why are the energy-rich substances produced by oxidation in the whole stem utilized only by the ends of the stem? Obviously, there must be an inhibitor in the rest of the stem which blocks the utilization of these substances. The presence of an inhibitor was suggested by Rose and Rose (1941) as an explanation for the failure of regeneration at ligatured ends and at ends capped with glass tubes. In a stem with both ends cut the inhibitor will escape from the cells at the cut ends but will remain in the cells between the cut ends. Therefore, although the O_2 consumption and energy production of the cells in the middle of the stem are high enough for regeneration, the process is blocked by the accumulation of an inhibitor. The experiments of Miller (1942), Zwilling (1939), and Goldin and Barth (1941) are also consistent with the idea that the stem produces an inhibitor which must diffuse out before regeneration can occur.

There is evidence that oxygen is antagonistic to this inhibitor, since high concentrations of oxygen can stimulate ligatured stems, in which the inhibitor accumulates, to begin regeneration (Rose and Rose, 1941). Likewise, vigorous shaking in the Warburg flasks will cause regeneration in ligatured stems (Barth, 1940*a*). Finally, Goldin (1942*b*) has shown that the inhibition of regeneration by acid can be overcome with high O_2 concentrations.

Summarizing, then, the suggestion is made that the process of regeneration is controlled by a balance between the concentration of oxygen and the concentration of inhibitors produced by the stem. Beginning at the cut end of the stem, where the inhibitor escapes by diffusion, the tissue will regenerate for a certain distance (1–2 mm.) proximal to the cut surface. At this level the tissues have a concentration of the inhibitor which will just block regeneration. An increase in the concentration of oxygen will antagonize the inhibitor, allowing more tissue to partake in the process of regeneration, and the threshold of inhibition is pushed more proximally.

III. ANTAGONISM BETWEEN OXYGEN AND URETHANE

If the preceding argument is correct, it might be expected that oxygen would release the inhibition of regeneration exercised by an externally applied inhibitor. Ethyl urethane was used in various concentrations in sea water at high con-

centrations of oxygen and found to have no effect on regeneration in concentrations from M/10,000 to M/100 (see Table 2). The small variations in size and time are found in all experiments.

TABLE 2

EFFECT OF ETHYL URETHANE ON REGENERATION

Stems 7–8 mm. in length; 120 stems used for experiment; concentration of oxygen, 18.8 cc. per liter.

Urethane Concentration (Molar)	Length (Mm.)	Time (Hours)
0	1.96	32.1
0.0001	1.96	31.6
0.003	2.00	32.1
0.001	1.96	34.8
0.002	1.97	31.6
0.01	2.12	34.1

However, at higher concentrations of ethyl urethane and lower concentrations of oxygen, inhibition is obtained, as shown in Table 3. These experiments compare the effects of various concentrations of urethane in 4.8 cc. of oxygen per liter with 16.6 cc. of oxygen per liter.

The results show that urethane reduces the size of the primordium and prolongs the time necessary for regeneration. Further, oxygen is able to overcome the inhibition by urethane. For example, at a concentration of 0.05 M, urethane reduces the primordium from 1.42 mm. to 0.98 mm. in an O_2 concentration of 4.8 cc. per liter. It thus prevents 0.44 mm. of the stem from regenerating. Increasing the O_2 concentrations to 16.6 cc. per liter not only permits this 0.44 mm. of the stem to regenerate but adds 0.37 mm. more to the primordium. Similar results are obtained with the time necessary for regeneration. Urethane of 0.025 M prolongs the time from 36.0 to 47.4 hours in 4.8 cc. of oxygen per liter. Increase of the O_2 concentration to 16.6 cc. per liter reduces the time back to 36.2 hours, just neutralizing the effect of urethane.

The important point to be made here is that tissue which is unable to regenerate because of the presence of an inhibitor can be stimulated to do so by increasing the concentration of oxygen. Since the presence of natural inhibitors in the stem has been demonstrated by several investigators, we may draw the conclusion that oxygen increases the amount of stem forming the primordium by shifting the threshold for inhibition. The cells proximal to the base of the primordium are thus pictured as inhibited with respect to regeneration, but with increase in concentration of oxygen this proximal region is released from inhibition and is added to the primordium. This hypothesis calls for a gradient in the concentration of the inhibitor such that it is present in lowest concentration at the cut end of the stem. As we proceed away from the cut, the concentration of the inhibitor increases, reaching the threshold value.

TABLE 3

RELEASE OF URETHANE INHIBITION BY OXYGEN

Stems 5–7 mm. in length; 240 stems used; temperature, 18.5° C.; length of primordium and time for regeneration compared at 4.8 cc. of oxygen per liter and 16.6 cc. of oxygen per liter.

Urethane Concentration (Molar)	Length (Mm.)		Time (Hours)	
	4.8 cc. O_2	16.6 cc. O_2	4.8 cc. O_2	16.6 cc. O_2
0	1.42	1.92	36.0	30.0
0.006	1.30	1.94	46.5	31.4
0.013	1.35	1.90	45.6	31.4
0.025	1.35	1.96	47.4	36.2
0.05	0.98	1.79	59.0	44.5
0.1	0.00	0.00	.....	.....

The simplest situation would be one in which the inhibitor could readily diffuse out of cells at the cut surface but not so readily out of other parts of the stem. A diffusion gradient would be set

up at the end which would give the required gradient in concentration of the inhibitor, as shown in Figure 1. Thus, at an O_N concentration of oxygen the length of the hydranth is L_N with the threshold of inhibition at I_N. An increase of oxygen to O_{N+1} would shift the threshold of inhibition to I_{N+1}, and the length of the primordium would be L_{N+1}. Similarly, a decrease of oxygen to O_{N-1} would shift the threshold of inhibition to I_{N-1} and the length to

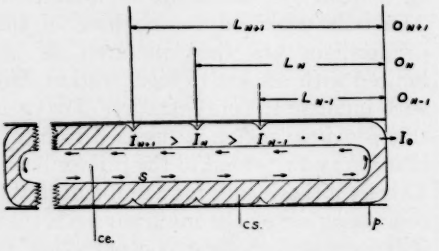

FIG. 1.—Schema showing lengths of hydranths at three concentrations of oxygen. L_N at O_N; L_{N-1} with diminished concentration of oxygen O_{N-1}; and L_{N+1} with increased concentration of oxygen O_{N+1}. A hypothetical inhibitor (I) in the tissue of the coenosarc is illustrated as diffusing out of the cut end. This diffusion sets up an inhibitor gradient as indicated by $I_{N+1} > I_N > I_{N-1} \ldots . I_0$. S = substances circulating in coelenteron; *ce.* = coelenteron; *cs.* = coenosarc; *p.* = perisarc.

L_{N-1}. This treatment is more in accord with the facts than the hypothesis presented earlier (Barth, 1940*b*). The following facts are consistent under this new hypothesis.

1. Regeneration occurs only at cut ends or where the perisarc has been removed (Zwilling, 1939; Nakamura, 1939). Loss of the inhibitors from the exposed cells by diffusion lowers the concentration for some distance from the exposed surface, thereby allowing regeneration.

2. A ligature of the perisarc over the cut surface blocks regeneration. The diffusion of the inhibitors is retarded by the perisarc.

3. A glass cap over the cut end blocks regeneration. The inhibitors soon accumulate, and the concentration at the cut surface remains too high for regeneration.

4. Regeneration is reduced when a glass tube is placed around the stem (Miller, 1942). Escape of inhibitors through the perisarc is retarded, increasing the internal concentrations, which reduces the regeneration at the ends.

5. Ligatured stems in which regeneration is blocked may be stimulated to regenerate by continuous shaking. The inhibitors are removed faster by shaking, and the internal concentration is less.

6. Circulation of sea water increases regeneration when compared with standing sea water (Miller, 1937). The inhibitors are removed more rapidly with circulation.

7. Explants of stem regenerate only at a free surface (Child, 1928; Goldin, 1942*a*). At any attached surface the inhibitor is not able to diffuse out of the tissues.

8. Lowered temperature results in larger primordia (Moog, 1941). If it is assumed that the diffusion of the inhibitor has a Q_{10} of 1.3 while the reactions leading to the formation of the inhibitor have a Q_{10} of 2.0, then a lowering of the temperature will result in relatively less inhibitor at the ends because of the disparity in temperature coefficients. In connection with temperature effects, it should be noted that the time for regeneration is longer for the lower temperatures (Moore, 1910).

IV. TIME OF DETERMINATION OF THE LENGTH OF THE PRIMORDIUM

Since the concentration of oxygen determines the length of the hydranth, the time at which determination of length occurs can be studied. Thus, we

can start the stem at a high concentration of oxygen and then change at intervals to a low concentration. Similarly, stems can be started in low oxygen and be shifted to high oxygen.

Preliminary experiments in which oxygen or nitrogen was bubbled through sea water to give a high or low concentration of oxygen showed that the size of the primordia and the time for regeneration could be varied over wide limits (Table 4). For example, stems placed in N_2 sea water for varying lengths of time and then returned to O_2 sea water showed that the size of the primordium was progressively reduced as the sojourn of the stems in N_2 sea water was increased. During from 5 to 22 hours of exposure to N_2 sea water the stems appear to undergo no regeneration, since, when they are returned to O_2 sea water, they take the same time for regeneration as freshly cut stems.

Reversal of treatment so that stems are first exposed to O_2 sea water, followed by N_2 sea water at intervals, shows that the size of the primordium can be reduced in the later stages of regeneration. After 11 hours' exposure to O_2 sea water, followed by 14 hours in N_2 sea water, the primordium is reduced from 1.66 to 1.29 mm.

Following a number of preliminary experiments with oxygen and nitrogen, a thorough study of the determination of length and time was carried out. The methods will be given in some detail, including the complete data from one experiment. One hundred and seventy-six stems were prepared in the usual way and cut to 8 mm. in length. These stems were divided into six groups. Group No. 1 was placed in a 500-cc. glass-stoppered bottle filled with sea water at an O_2 concentration of 2.2 cc. per liter. The remaining five groups were placed in bottles at 13.0 cc. of oxygen per liter. Of these five groups, No. 2 was transferred to sea water at 2.2 cc. of oxygen per liter at 2.5 hours; No. 3 at 5.0 hours; No. 4 at 16.0 hours; No. 5 at 20.0 hours; and the last, No. 6, remained at 13.0 cc. of oxygen per liter. At the end of 23.5 hours all six groups were transferred to sea water at 13.0 cc. of oxygen per liter, and the time for the formation of the primordium was recorded for each stem along with its length and diameter.

TABLE 4

EFFECT OF LOW OXYGEN CONCENTRATIONS ON SIZE AND TIME FOR REGENERATION

Hours of Treatment		Regeneration	
		Length (Mm.)	Time (Hours)
N_2 Sea Water	O_2 Sea Water		
0	25	1.66	29.2
5	26	1.56	34.4
22	26	1.41	53.7
46	26	1.00	84.0
O_2 Sea Water	N_2 Sea Water		
25	0	1.66	29.2
11	14	1.29	32.2
5	20	1.21	30.0

All these operations were carried out in a constant-temperature bath at 18.5° C., and the data are recorded in Table 5, where the time (t) is in hours, and L and D are length and diameter in ocular micrometer units (1 unit = 40 μ). If a difference in length of two times the square root of the sum of the squares of the standard errors of the means of two groups is considered a significant difference, then a difference of 2.8 micrometer units, or 0.11 mm., is generally significant. In regard to the time for regeneration, all differences between groups Nos. 1, 2, 3, 4, and 5 are significant, while the difference between Nos. 5 and 6 is not. There are no significant differences in diameter, and this simply means that in

selection of the stems a random choice was made.

Table 6 presents a summary of all the data obtained. In Experiments 1, 2, 3, stems do not regenerate as well as freshly collected stems. In Experiment 7 the stems were exposed to low O_2 concentrations for much longer periods of time.

TABLE 5

VARIATION IN REGENERATION OF *Tubularia*

L = length of primordia in micrometer units; D = diameter of stem in micrometer units; t = time in hours; m = mean; σ = standard deviation; σm = standard error of the mean. See text for treatment of various groups.

Group No. 1 (30 Stems)			Group No. 2 (30 Stems)			Group No. 3 (28 Stems)			Group No. 4 (28 Stems)			Group No. 5 (31 Stems)			Group No. 6 (29 Stems)		
L	D	t	L	D	t	L	D	t	L	D	t	L	D	t	L	D	t
33	14	40.0	40	15	42.0	32	13	33.5	40	14	28.5	51	14	26.0	53	15	24.0
33	14	40.0	51	14	45.5	28	14	33.5	27	14	28.5	47	15	26.0	41	13	24.0
39	14	40.0	40	11	45.5	26	12	33.5	38	12	28.5	41	12	26.0	45	14	24.0
34	12	40.0	40	13	45.5	32	13	33.5	48	13	28.5	45	13	26.0	48	12	24.0
38	11	42.0	43	14	45.5	37	15	37.5	39	14	28.5	44	14	26.0	43	13	24.0
37	14	45.5	33	14	45.5	35	14	37.5	30	12	28.5	41	12	26.0	45	12	24.0
39	14	45.5	42	13	45.5	28	12	37.5	40	13	28.5	49	13	26.0	33	12	24.0
32	14	45.5	37	13	45.5	24	13	37.5	33	15	28.5	45	12	26.0	39	13	24.0
36	14	45.5	36	14	45.5	35	15	37.5	43	14	28.5	48	12	26.0	45	13	26.0
44	13	45.5	44	12	45.5	36	15	40.0	41	14	28.5	42	13	26.0	43	14	26.0
38	13	45.5	41	14	50.0	32	13	40.0	44	13	28.5	54	13	26.0	41	14	26.0
36	13	45.5	38	14	50.0	35	12	40.0	45	15	28.5	41	14	26.0	45	12	26.0
35	14	45.5	31	14	50.0	32	14	40.0	39	13	28.5	37	13	28.5	50	14	26.0
40	15	45.5	35	12	50.0	42	15	42.0	34	15	28.5	36	13	28.5	49	13	26.0
43	15	45.5	36	13	50.0	38	14	42.0	34	14	31.0	34	15	28.5	40	12	26.0
35	14	45.5	38	13	50.0	36	15	42.0	31	13	31.0	43	14	28.5	47	13	26.0
36	13	45.5	33	15	50.0	36	11	45.5	35	13	31.0	41	15	28.5	45	12	26.0
37	13	50.0	32	14	54.0	38	15	45.5	34	14	31.0	40	15	28.5	47	13	26.0
30	16	50.0	47	14	54.0	36	16	50.0	44	12	31.0	50	15	28.5	47	14	26.0
36	15	50.0	31	15	54.0	32	15	50.0	43	12	31.0	36	13	28.5	41	14	26.0
39	12	50.0	30	14	54.0	39	14	50.0	47	14	31.0	46	12	28.5	55	17	28.5
31	14	50.0	33	14	54.0	44	16	50.0	31	15	31.0	46	14	28.5	46	15	28.5
36	12	50.0	40	12	54.0	32	13	50.0	50	14	31.0	36	14	28.5	60	15	28.5
31	14	50.0	36	13	54.0	34	15	50.0	28	15	31.0	38	12	28.5	32	13	28.5
30	13	50.0	37	16	60.0	35	14	50.0	38	12	31.0	50	14	28.5	50	13	28.5
39	12	54.0	28	12	60.0	34	12	50.0	38	12	33.5	31	14	31.0	36	14	31.0
40	16	54.0	27	14	60.0	31	14	50.0	39	15	33.5	35	14	31.0	40	13	33.5
41	13	54.0	28	17	60.0	34	14	60.0	35	13	36.0	30	14	31.0	37	15	36.0
48	17	60.0	29	15	60.0	.....	.....	.....	.....	.....	.....	38	13	33.5	45	14	42.0
38	15	60.0	27	13	60.0	.....	.....	.....	.....	.....	.....	43	12	33.5	.....	.....	.....
.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	46	16	33.5	.....	.....	.....
m 36.8	13.8	47.6	36.1	13.7	51.3	34.0	13.8	43.3	38.1	13.5	30.1	42.0	13.5	28.3	44.4	13.5	27.2
σ 4.2	1.4	5.2	5.8	1.25	5.5	4.5	1.4	6.5	6.0	1.1	1.9	6.0	1.2	2.3	5.3	1.1	4.0
σm 0.76	0.25	0.95	1.06	0.23	1.0	0.85	0.26	1.2	1.1	0.20	0.36	1.1	0.22	0.42	1.0	0.20	0.74

and 4 the condition of the stems and the experiment were very similar. Experiment 5 should carry the special note that the stems were old and had very large gonophores. In Experiment 6 the stems were not used when collected but had been in the laboratory for 2 days. These

The conclusions from these data are as follows:

1. The size of the primordium can be altered during the process of regeneration. Even after about 19 hours, or approximately 70 per cent of the average time necessary for regeneration, the

TABLE 6

DETERMINATION OF THE HYDRANTH IN *Tubularia*

Treatment with high O_2 followed by low O_2. After treatment, stems returned to high O_2 for completion of regeneration. L = length of primordium of hydranth in millimeters; t = time from cutting to formation of primordium in hours; "Delay" = number of hours delay compared with control at high O_2 continuously.

Oxygen Concentration	Treatment (Hours)		L (Mm.)	t (Hours)	Delay (Hours)	Col. 3 *minus* Col. 6	Remarks on Stems
(1)	High O_2 (2)	Low O_2 (3)	(4)	(5)	(6)	(7)	(8)
Expt. 1: high O_2 = 13.9 cc. per liter; low O_2 = 1.2 cc. per liter	23.5	0.0	1.78	27.2	0	0	Length, 8 mm.; 176 stems; condition good
	20.0	3.5	1.68	28.2	1	+ 2.5	
	16.0	7.5	1.52	30.1	2.9	+ 4.6	
	5.0	18.5	1.36	43.3	15.9	+ 2.6	
	2.5	21.0	1.44	51.3	24.1	− 3.1	
	0.0	23.5	1.47	47.7	20.5	+ 3.0	
Expt. 2: high O_2 = 18.5 cc. per liter; low O_2 = 2.5 cc. per liter	24.0	0.0	1.72	30.0	0	0	Length, 6–7 mm.; 198 stems; condition good
	19.5	4.5	1.54	33.2	3.2	+ 1.3	
	16.0	8.0	1.37	36.2	6.2	+ 1.8	
	5.0	19.0	1.48	50.0	20.0	− 1.0	
	2.5	21.5	1.38	52.0	22.0	+ 0.5	
	0.0	24.0	1.52	51.6	21.6	+ 2.4	
Expt. 3: high O_2 = 15.0 cc. per liter; low O_2 = 1.2 cc. per liter	24.0	0.0	1.91	25.0	0	0	Length, 6 mm.; 150 stems; condition good
	18.5	5.5	1.85	28.1	3.1	+ 2.4	
	14.0	10.0	1.47	34.0	9.0	+ 1.0	
	5.0	19.0	1.55	48.4	23.4	− 4.4	
	2.0	22.0	1.60	48.0	23.0	− 1.0	
	0.0	24.0	1.60	48.1	23.1	+ 1.0	
Expt. 4: high O_2 = 19.8 cc. per liter; low O_2 = 1.0 cc. per liter	23.5	0.0	1.74	27.9	0	0	Length, 6 mm.; 180 stems; condition good
	19.0	4.5	1.58	31.6	3.7	+ 0.8	
	14.0	9.5	1.51	35.2	7.3	+ 2.2	
	5.0	18.5	1.48	42.0	14.1	+ 4.4	
	2.0	21.5	1.40	48.6	20.7	+ 0.8	
	0.0	23.5	1.55	49.2	21.3	+ 2.2	
Expt. 5: high O_2 = 15.0 cc. per liter; low O_2 = 1.2 cc. per liter	24.0	0.0	2.18	25.4	0	0	Length, 15 mm.; 130 stems; old stems with large gonophores
	18.5	5.5	2.02	30.2	4.8	+ 0.7	
	14.0	10.0	1.65	38.0	12.6	− 2.6	
	5.0	19.0	1.79	51.5	26.1	− 7.1	
	2.0	22.0	1.98	49.2	23.8	− 1.8	
	0.0	24.0	1.95	50.6	25.2	− 1.2	
Expt. 6: high O_2 = 13.8 cc. per liter; low O_2 = 1.3 cc. per liter	23.0	0.0	1.61	27.0	0	0	Stems in laboratory 2 days; 132 stems; length, 5–6 mm.
	5.0	18.0	1.26	54.7	27.7	− 9.7	
	2.0	21.0	1.10	58.9	31.9	−10.9	
	0.0	23.0	1.18	60.1	33.1	−10.1	
Expt. 7: high O_2 = 13.9 cc. per liter; low O_2 = 1.2 cc. per liter	23.5	0.0	1.77	25.5	0	0	Long exposure to low O_2; 92 stems; length, 5–6 mm.
	5.0	67.5	1.22	104.0	78.5	−11.0	
	2.5	70.0	1.26	107.0	81.5	−11.5	
	0.0	72.5	1.24	103.0	77.5	− 5.0	

primordia are significantly shorter in Experiments 2, 4, and 5. In Experiments 1 and 3 the primordia are smaller, but the differences are not significant.

2. After from 5 to 14 hours of regeneration at high O_2 concentrations, followed by treatment with low O_2 concentrations, the primordium size is minimum. This may indicate that the size of the primordium is determined after this period and therefore is fixed under the influence of the low O_2 concentration. Peebles (1931) found that after 15 hours of regeneration the primordium could no longer be reduced in size by making the stem shorter. Between 6 and 13 hours some of the primordia could be reduced in size, while after from 2 to 4 hours the size of all of the hydranths was reduced. This indicates no determination during 2–4 hours of regeneration, progressive determination from 6–13 hours, and complete determination at 15 hours.

3. Regeneration is almost completely inhibited when the stems are placed in low O_2 concentrations immediately after cutting (see Table 6, 0 hours at high oxygen.) When returned to high O_2 concentrations, they form, after 24 hours, primordia which are larger than those of stems which were treated with high oxygen for 5–14 hours. One interpretation of this increased size is that little or no regeneration takes place at low O_2 concentrations, so that the size is determined after the stems have been returned to high O_2 concentrations. In the 5–14-hour groups, regeneration starts at high oxygen; and, when the stems are then changed to low oxygen, determination of size takes place.

4. The delay in time for regeneration is approximately the time during which the stems were in low oxygen. In Table 6, column 6 gives the delay, while column 7 gives the difference between the time the stems were treated with low oxygen and the delay. A positive value in column 7 means that some regeneration took place in the low oxygen. Zero means that no regeneration occurred at low oxygen (or that the amount of regeneration at low oxygen was equal to a delay at high oxygen). A negative value means that stems were delayed even during the period in high oxgyen. In general, young stems in healthy condition show positive values, i.e., manage to undergo some regeneration in low oxygen (Expts. 1, 2, 3, and 4). In Experiment 5 old stems were used, and they appear to be more susceptible to low oxygen, showing retardation even while in high oxgyen. Similarly, in Experiment 6 young stems were kept in the laboratory for 2 days and thus are not in optimum condition. The negative values in Experiment 7 are the result of a longer treatment (67.5–72.5 hours) of the stems with low oxygen, so that they do not recover immediately on change back to high oxygen.

The results show that determination of the length of the primordium is progressive with time. There is no evidence of a sudden determination at some definite time. On the contrary, it is possible to change the length of the primordium during most of the period of regeneration. This implies that the distal parts of the hydranth are determined first and the more proximal parts later, for the changes in length of the primordium mean a change in the amount of stem derived from the proximal regions. Obviously, this proximal region forming the base of the hydranth must remain undetermined until the final few hours of regeneration.

The determination of the hydranth which occurs during the first few hours, as shown by the reduction of methylene blue in *Corymorpha* (Child and Watanabe, 1935; Watanabe, 1935), may be a labile determination. When the condi-

tions are changed by altering the O_2 concentration, a redetermination may take place because a new ratio between an internal inhibitor and oxygen concentration is established. This redetermination may be simply an increase in the length of the primordium caused by a proximal shift of the level of constriction; or it may be that all the parts of the hydranth are redetermined, so that normal proportions between the parts are maintained. Measurements of the length of the proximal and distal tentacular regions would decide between these two possibilities. Child (1931), using potassium cyanide, showed that the normal proportions between the parts of the hydranth were changed by this inhibitor, often resulting in partial forms.

TABLE 7

TIME OF ACTION OF URETHANE ON SIZE OF REGENERANT AND TIME FOR REGENERATION

Concentration, 0.07 M in sea water of O_2 tension of 16.5 cc. per liter; 112 stems, 7–8 mm. in length.

Period Treated (Hours)	Regeneration		Delay (Hours)
	Length (Mm.)	Time (Hours)	
0.....	2.20	33.6	.....
0–10.....	1.72	47.3	13.7
10–20.....	1.76	51.0	17.4
20–30.....	1.62	38.0	4.4

Similar results were obtained with urethane treatment at different intervals during regeneration (Table 7). Here it is found that the hydranth size could be reduced with 0.07 M urethane applied at various times. The greatest reduction occurs when the stems are treated from the twentieth to the thirtieth hour of regeneration.

The period of greatest effect of urethane on the time for regeneration is the 10–20-hour interval when a 10-hour treatment with urethane retards regeneration 17.4 hours. On the other hand, a 10-hour treatment from 20 to 30 hours gives only a 4.4-hour delay, indicating that 6.6 hours of regeneration took place in urethane.

Thus, size of primordium and time for regeneration seem to be controlled by factors operating at different times. A maximum effect on size but a minimum effect on time is obtained by treatment with urethane from 20 to 30 hours.

V. SUMMARY

The effects on *Tubularia* of increasing the concentration of oxygen in sea water are (1) an increase in the amount of oxygen consumed by the stem, (2) an increase in the size of the regenerant, and (3) a decrease in the time required for regeneration. The increase in size (length) is explained on the basis of a proximal shift in the threshold of inhibition, thus allowing more of the proximal inhibited region of the stem adjacent to the base of the regenerant to partake in regeneration (Fig. 1).

The proximal shift in the threshold of inhibition was studied by the use of ethyl urethane to produce inhibition of the proximal region of the regenerant and the use of high concentrations of oxygen to shift or release this inhibition. Urethane treatment results in shorter hydranths by preventing the stem adjacent to the base of the regenerant from being incorporated into the regenerant. When oxygen is used, this inhibited region of the stem is released and the resulting hydranths are longer.

The progressive determination of the size of the regenerant was studied by exposing the stems first to high concentrations of oxygen and changing the stems to low concentrations at various intervals. The results show that the size of the

regenerant can be reduced by low concentrations of oxygen even in the late stages of regeneration. Thus the parts of the hydranth (at least the basal region) are not completely determined until more than 70 per cent of the total time for regeneration has elapsed.

The time required for regeneration was altered by oxygen and by urethane. Low concentrations of oxygen completely inhibit regeneration without injuring the stems, so that they regenerate in about the normal time when removed to high concentrations of oxygen. Urethane is most effective when applied during the 10–20-hour interval of regeneration.

LITERATURE CITED

BARTH, L. G. 1938*a*. Oxygen as a controlling factor in the regeneration of *Tubularia*. Physiol. Zoöl., **11**:179–86.

———. 1938*b*. Quantitative studies of the factors governing the rate of regeneration in *Tubularia*. Biol. Bull., **74**:155–77.

———. 1940*a*. The relation between oxygen consumption and rate of regeneration. *Ibid.*, **78**: 366–74.

———. 1940*b*. The process of regeneration in hydroids. Biol. Rev., **15**:405–20.

———. 1944. Colloid chemistry of development, in Alexander's Colloid chemistry, Vol. V: Theoretical and applied, pp. 851–59. New York: Reinhold Pub. Co.

CHILD, C. M. 1928. Axial development in aggregates of dissociated cells from *Corymorpha palma*. Physiol. Zoöl., **1**:419–61.

———. 1931. Experimental modification of the scale of organization in the reconstitution of *Tubularia*. *Ibid.*, **4**:165–87.

———. 1941. Patterns and problems of development. Chicago: University of Chicago Press.

CHILD, C. M., and WATANABE, Y. 1935. Differential reduction of methylene blue by *Corymorpha palma*. Physiol. Zöol., **8**:395–416.

GOLDIN, A. 1942*a*. Factors influencing regeneration and polarity determination in *Tubularia crocea*. Biol. Bull., **82**:243–54.

———. 1942*b*. A quantitative study of the interrelationship of oxygen and hydrogen ion concentration in influencing *Tubularia* regeneration. *Ibid.*, **82**:340–46.

GOLDIN, A., and BARTH, L. G. 1941. Regeneration of coenosarc fragments removed from the stem of *Tubularia crocea*. Biol. Bull., **81**:177–89.

KALCKAR, H. M. 1942. The role of phosphate in cellular assimilations. Biol. Rev., **17**:28–45.

MILLER, J. A. 1937. Some effects of oxygen on polarity in *Tubularia crocea*. Biol. Bull., **73**:369 (abstract).

———. 1939. Experiments on polarity determination in *Tubularia* regenerates. Anat. Rec., Suppl., **75**:38–39.

———. 1942. Some effects of covering the perisarc upon *Tubularia* regeneration. Biol. Bull., **83**: 416–27.

MOOG, F. 1941. The influence of temperature on reconstitution in *Tubularia*. Biol. Bull., **81**:300–301.

MOOG, F., and SPIEGELMAN, S. 1942. Effects of some respiratory inhibitors on respiration and reconstitution in *Tubularia*. Proc. Soc. Exper. Biol. and Med., **49**:392–95.

MOORE, A. R. 1910. The temperature coefficient for the process of regeneration in *Tubularia crocea*. Arch. f. Entw'mech., **29**:145–48.

NAKAMURA, NORIS. 1939. Formation of hydranth in *Tubularia* at the place whence perisarc has been removed. Zoöl. Mag., **51**:183–84.

PEEBLES, F. 1931. Some growth-regulating factors in *Tubularia*. Physiol. Zoöl., **4**:1–35.

ROSE, S. MERYL, and ROSE, F. C. 1941. The role of a cut surface in *Tubularia* regeneration. Physiol. Zoöl., **14**:328–43.

WATANABE, Y. 1935. Physiological dominance in *Corymorpha palma* in relation to reconstitution and methylene blue reduction Physiol. Zoöl., **8**:417–41.

ZWILLING, E. 1939. The effect of removal of perisarc on regeneration in *Tubularia crocea*. Biol. Bull., **76**:90–103.

THE EFFECTS OF ETHER UPON THE DEVELOPMENT OF DROSOPHILA MELANOGASTER

IVOR CORNMAN[1]

THE effects of ether upon *Drosophila* embryos are of particular interest because ether is used for anesthetizing the flies during the course of many types of experiments and because narcotics have been shown to have deleterious effects upon dividing cells. The possibility of producing polyploid *Drosophila* provides an additional incentive.

Two types of data were collected. One is cytological, obtained from the study of sectioned eggs fixed at successive times after treatment. The other consists of statistical records of mortality, retardation of development, and abnormality of surviving adults.

MATERIALS AND METHODS

A stock of Oregon-R wild-type *Drosophila* inbred for more than fifty generations was used throughout the investigation. The normal embryology of this race has been thoroughly studied (Huettner, 1924; Poulson, 1937; Rabinowitz, 1941), and it has been extensively used for experimentation. The adult flies from which eggs were obtained were never etherized. Rather, they were sorted and transferred by means of a small aspirator, such as is used in the field to collect minute insects.

The high vapor pressure of ether makes measurement of small quantities of the liquid difficult, and so in these experiments the vapor was measured by volume. To supply ether vapor, a 500-cc. flask containing liquid ether was kept in a water bath maintained at 39° ± 1° C. by an ordinary thermostatically controlled aquarium-heater. Such a flask is large enough to maintain a reserve of vapor, and the water bath stabilizes the temperature. This generator was closed by a stopcock and attached by a short tube to the etherizing chamber. The chamber was a cylindrical tube large enough to admit a 1 × 3-inch slide and was supplied at each end with stopcocks. One stopcock connected with the generator; the other, with a 20-cc. all-glass syringe. The amount of ether admitted to the chamber was measured by the amount of air displaced into the syringe. The plunger was light enough and moved freely enough to make its effect on the pressure negligible. The dose used throughout the experiments was 20 cc. of ether in the 60-cc. chamber, giving a mixture one-third ether and two-thirds air by volume.

Eggs of known age were collected by timing the eggs laid by groups of five to ten females which had been without food overnight and had mated during the morning of the experiment. Strips of blotting paper of a size conveniently fitting on a glass slide were soaked in heavily yeasted water containing a few pieces of banana. The flies were allowed

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to lay eggs on the paper for 1 hour to eliminate eggs of uncertain age. The paper strips were then changed every 15 minutes. They were used only if the number of eggs on them was large enough to indicate that the eggs had been laid in quick succession, and therefore were of known age, since only one egg at a time is fertilized by the female. For the developmental studies the position of each egg was charted in order that its time of hatching could be recorded with certainty.

The eggs were divided into five sets, four for treatment at successively later stages of development (A–D) and a control set (E). Most of the eggs were etherized at an early stage, so that the treatment would catch the earliest cleavages, producing a more general effect and increasing the chances of obtaining complete polyploidy. This exposure to ether, in set A, was begun 5 minutes after the eggs were removed from the laying vial and continued for 20 minutes. The eggs were thus anywhere from 5 to 20 minutes old when the etherization was begun, and 25 to 40 minutes old at the termination. During this period of time the earliest-laid egg, under normal conditions, would have proceeded as far as the third cleavage, having passed through the first two cleavages of the zygotic nucleus during the time encompassed by the ether exposure (Rabinowitz, 1941). The other 20-minute ether treatments began at (set B) 65, (set C) 85, and (set D) 125 minutes after the end of the egg-laying period. These times concide with the migration of the nuclei from the yolk cylinder to the peripheral cytoplasm (set B), with pole (germ) cell formation (set C), and with invagination of the proctodeum (set D).

For cytological study the eggs were punctured in Bouin's or formol-alcohol-acetic fixative at varying times after etherization. In properly fixed eggs the results were identical with both fixatives. Sections, usually 8 μ thick, were stained in Heidenhain's iron haematoxylin, gentian violet, or by the Feulgen nucleal reaction. Altogether, 267 eggs were sectioned for study: 187 treated and 80 control.

For developmental study the young were raised at 25° ± 0.5° C. The paper carrying the eggs was transferred from the etherizing chamber to a 30 × 100-mm. shell vial with $\frac{1}{2}$ inch of banana-agar food in the bottom. The number of eggs hatched was recorded each hour. Only when a larva was completely free from the chorion was it counted as hatched. Many of the eggs did not hatch, and these were fixed for cytological examination. As each fly pupated, it was transferred to a separate vial, so that pupation and emergence time (recorded every 3 hours) could be kept for the individual fly. Records were kept of abnormalities of the emerged imagos and of the approximate stage to which unemerged flies had developed. The data include 275 control eggs and 566 treated.

CYTOLOGICAL OBSERVATIONS

The normal embryology of *Drosophila* has been worked out in detail by Huettner (1923, 1924, 1933), Poulson (1937), and Rabinowitz (1941), and so the normal is not described here except to make clear the ways in which certain effects of ether produce variance from the normal. In addition to the eggs used as control for these experiments, the large series of Dr. A. F. Huettner's slides were available for study.

In general, the visible abnormalities increased in severity with increase in time between etherization and fixation and decreased with increase in age at which eggs were etherized. There were no abnormalities in the 80 untreated eggs.

I. CHROMATIN ABNORMALITIES

Ether caused chromosomes and chromatin granules to fuse. This fusion of chromatin was in some cases partial, producing nuclei containing amorphous granular masses (Pl. I, Fig. 3, *B*, *C*), and in others complete, producing typical pycnotic nuclei (Pl. I, Figs. 3, *D* and *E*; 4; 7). Some chromatin masses of this sort lay free in the cytoplasm, lacking a nuclear membrane. Chromosomes often fused partially, producing clumps (Pl. I, Fig. 2) when fusion was considerable, or chromatin bridges when the chromosomes still responded to the influence of the anaphase spindle but could not separate normally (Pl. I, Fig. 1). There were 60 eggs (32.1 per cent) showing such abnormalities.

This fusion represents a fairly generalized response of both plant and animal cells to ether and many other chemical and physical agents (cf. Politzer, 1934). Two of the many investigations with ether can be mentioned: that of Rosenfeld (1932) with chick-embryo fibroblasts and that of Hocquette and Villard (1932) with *Raphanus* seedlings.

Differences in quantity of chromatin in the nuclei were striking, even within the same eggs. The series in Plate I, Figure 3, *B*–*F*, are all from one etherized egg (in a normal egg of this age, nuclei are of identical size); a degenerating yolk nucleus (*A*) from a normal egg is included for comparison. There were marked discrepancies between the chromatin masses mentioned above and the apparent volume of chromosomes in mitotic figures in the same egg. Such chromatin aggregations do not necessarily involve degeneration, if we may judge from giant nuclei which do not become pycnotic (Pl. I, Fig. 8) and from multipolar spindles with many times the normal chromosome number (Pl. I, Fig. 5). Clearly, there has been a duplication of chromatin, but no indisputably eupolyploid egg was found. There were 8 eggs showing increased chromatin (4.3 per cent), as indicated by large nuclei, giant chromatin masses, or polyploid spindles.

Ether has been reported to produce chromosome doubling in chick fibroblasts (Rosenfeld, 1932); and Pincus and Waddington (1939) obtained large nuclei in rabbit eggs after ether treatment, although they are cautious about classing them as polyploid.

II. SPINDLE ABNORMALITIES

Multipolar spindles with three to seven centrioles have been observed in the etherized eggs. Some tripolar spindles appeared to have the normal diploid chromosome number (Pl. I, Fig. 6), but the larger multipolar spindles (Pl. I, Fig. 5) were always associated with polyploidy. In such multipolar spindles the chromosomes lacked any regular orientation. There was some evidence that spindles were altered in shape, as well as in polarity, becoming blunt, barrel-shaped, and even rectangular or circular. Extreme degeneration of the spindle reduced it to an irregular area around the chromosomes, distinguishable from the cytoplasm only by its greater homogeneity. In such degenerated spindles the chromatin was usually pycnotic and amorphous. In 18 eggs (9.6 per cent), one or more abnormal spindles were found.

Wilson (1901), using ether, produced multipolar spindles, associated with suppressed cleavage and increased chromosome number, in sea-urchin eggs.

Multipolar spindles are the only important abnormality occurring to a significant degree in untreated *Drosophila* eggs (Huettner, 1927). None, however, were found in the 80 eggs serving as controls for these experiments. Huettner

(personal communication) estimated the frequency in presumably normal eggs to be one in 300 eggs, whereas in the etherized eggs of these experiments the frequency was five in 187 eggs (2.7 per cent). This raises the question as to whether the rare multipolar spindles in the untreated eggs may not also have been an ether effect, inasmuch as the parents had been etherized.

III. CYTOPLASMIC ABNORMALITIES

The distribution of cytoplasm was markedly influenced by ether. Normally each nucleus is surrounded by a small protoplasmic island while it is in the interior yolk column. In treated eggs the amount of cytoplasm comprising the islands varied from a thin layer enveloping a nucleus to areas two or three times the normal amount. One island around the polar body extended along half the length of the egg. Vacuoles often appeared in such islands, especially next to the nucleus (Pl. I, Figs. 2, 5). In about 15 per cent of the eggs the cytoplasm was definitely abnormal.

Apparently, the yolk spheres were also affected by ether. Instead of being round in outline, they were often fusiform, bottle-shaped, rodlike, or even stellate. Fusiform yolk bodies occur in untreated eggs, but not in such large numbers, nor in so many eggs. A quantitative estimate of the extent of yolk-sphere deformation was not attempted.

IV. KINEMATIC ABNORMALITIES

Nuclei were often observed to divide asynchronously, especially when other abnormalities were present. Further evidence of asynchronous division was obtained from counts of nuclei in early cleavages, where the nuclei should equal some power of 2. In one egg 85–100 minutes old, for instance, 20 cleavage figures were counted. In a normal egg of the same age there should be at least 128 figures; or, if the nuclei had divided synchronously at a retarded rate, the number should have been 32 or 16. Ten cases of asynchrony were found.

In 10 eggs ether also interfered with the normal migration of the nuclei from the yolk column to the periphery. In one egg, old enough to have reached the blastoderm stage, the nuclei were fewer than normal, and there were no more of them at the periphery than throughout the center. In cases where the number of nuclei was normal for the age of the egg, they did not migrate to the periphery at the usual time (between the seventh and eighth cleavage).

V. DEGENERATIVE CHANGES

The length of time an egg survived abnormalities varied considerably. Development after treatment proceeded no further than a few cleavages in some cases, while in others development continued to maturity, the abnormality being evidenced only by retardation of development or abnormality of the imago, as described in the second section.

An untreated sterile egg degenerates at 6–8 hours (Sonnenblick, unpublished). This degeneration in both abnormal and virgin eggs is a separation of the cytoplasm from the yolk and fat to form compact irregular spheres. When large numbers of nuclei were present in treated eggs, they sometimes formed undifferentiated aggregates in parts of the egg, while other areas of the cytoplasm broke down (Pl. I, Fig. 4). This abortive development and disintegration is similar to that found by Sonnenblick following X-radiation (1940).

For a long time the chromatin retained its typical staining reactions, regardless of the morphological alterations it had undergone; but in eggs 36 hours old (15 hours beyond hatching

time), a beginning of lysis of chromatin occurred, reducing the chromosomes and granular nuclei to amorphous globules, still Feulgen-positive.

Inasmuch as such disintegration of cytoplasm and chromatin was not found in fertilized eggs younger than 21 hours, it presumably does not represent a direct effect of ether but is more probably a nonspecific post-mortem change.

OBSERVATIONS ON MORTALITY AND RATE OF DEVELOPMENT

Flies that were allowed to continue developing after etherization showed lower viability, slower development, and morphological abnormalities. The differences in distribution of these effects showed that they occurred, to some extent, independently of each other.

I. MORTALITY

The sums of the mortality percentages from Table 1 are: set A, 63.2; set B, 46.9; set C, 22.9; set D, 26.4; set E, 16.4. These figures show that lethal effects of ether treatment were highest in the eggs which were youngest and in those eggs in which there was the most active nuclear migration at the time of treatment. Set A, etherized during the early cleavages, and set B, etherized during migration of the cleavage nuclei, combine these effects on the early, as well as the visibly active, stages. The mortality of set D, although only slightly higher than that of set C, is worth notice because in set D the etherization took place during germ-band elongation and proctodeal invagination, whereas ether reached the eggs of set C when the only visible activity was the budding of the germ cells (which are not necessary for the life of the individual).

II. RATE OF DEVELOPMENT

Etherized *Drosophila* developed more slowly than did the untreated. The delay, in terms of percentage, was about equally divided between the embryonic and the larval stages and became negligible during the pupal stage (Table 2). Only in set B, etherized during blastoderm anlage formation, was there a significant delay during the pupal stage.

III. DISTRIBUTION

The time of hatching of the treated eggs was distributed along a symmetrical normal curve. At pupation and emergence, however, the curves were skewed to the right by disproportionate retardation of some individuals. Some were so excessively delayed, scattered at points 15 σ and 20 σ beyond the mean, that they were omitted from the calculation of the statistics, since their inclusion would have given a distorted picture of the curve. Data for normal flies at hatching, pupation, and emergence give symmetrical distribution curves (Harnly, 1929). As with treated flies, the spread of the distribution curve becomes greater with each successive stage, when the number of individuals is plotted against time.

It is true for treated and untreated eggs that there was only rough correlation between time of pupation and time of emergence. However, when there was marked delay in pupation of etherized flies, there was also marked delay in emergence: A group of 7 retarded flies in set A, clearly separate from the main group, all pupated and emerged 1.5 σ or more beyond the mean.

Where there were enough flies to give a distribution of significant numbers at some distance from the mean (sets A and C), it was possible to show a decidedly different mortality rate in the parts of the curve (Table 3). In the treated flies the mortality of the delayed individuals was about five times that of the ones pupating near the mean, whereas the

TABLE 1

MORTALITY AND THE STATISTICS OF DEVELOPMENTAL RATE

Sets A–D were etherized for 15 minutes in an atmosphere one-third ether, beginning at progressively greater times after the end of the 15-minute egg-laying period: set A, 5 minutes; set B, 65 minutes; set C, 95 minutes; set D, 125 minutes. Set E represents the untreated eggs. σ_M = standard error of the mean. Difference of the means refers to the difference between control (set E) and the treated set. σ_d = standard error of this difference. Some individuals (col. 7) included in the mortality calculations could not be included in the calculation of delay. Most of these are in Part I, since there sometimes was doubt as to the exact time of the hatching of an egg, but that larva could not then be discarded. During pupation and emergence there were extremely delayed individuals which were omitted from N because their inclusion gave a mean value not representative of the average trend (cf. text). When there was any possibility that a pupa had been damaged in transferring, it was discarded (Part III, col. 3).

Set	Mortality					Delay								
	Total	Discarded	Died	Mortality (Per Cent)	Survived	Omitted from N	N	Mean Time (Hours)	σ_M	σ	Difference of Means	σ_d	Diff. Means / σ_d	Percentage of Difference
	I. Time to Hatching													
A	225	0	90	40.00	135	1	134	20.92	0.13	1.52	1.16	0.17	6.82	5.87
B	74	0	15	20.27	59	3	56	21.18	0.27	2.00	1.42	0.29	4.90	7.19
C	180	0	20	11.11	160	2	158	20.70	0.08	1.03	0.94	0.13	7.23	4.76
D	88	0	8	9.09	80	4	76	20.29	0.16	1.36	0.53	0.19	2.79	2.68
E	286	0	26	9.09	260	7	253	19.76	0.10	1.65	.....	.....	.....	.....
	II. Time to Pupation													
A	135	0	20	14.82	115	0	115	108.85	0.73	7.77	7.69	0.79	9.73	7.60
B	59	0	13	22.03	46	0	46	107.52	1.17	7.94	6.36	1.21	5.26	6.29
C	160	0	13	8.13	147	1	146	106.50	0.54	6.57	5.34	0.63	8.48	5.28
D	80	0	10	12.50	70	1	69	104.71	0.90	7.45	3.55	0.95	3.74	3.51
E	253	0	15	5.93	238	1	237	101.16	0.32	4.86	.....	.....	.....	.....
	III. Time to Emergence													
A	115	4	8	7.21	103	0	103	202.02	0.70	7.14	8.28	0.84	9.86	4.27
B	46	2	2	4.55	42	0	42	202.86	1.29	8.34	9.12	1.37	6.66	4.71
C	147	9	5	3.62	133	2	131	198.86	0.47	5.42	5.12	0.66	7.76	2.64
D	70	8	3	4.84	59	1	58	197.66	0.64	4.86	3.92	0.79	4.96	2.02
E	238	25	3	1.41	210	0	210	193.74	0.46	6.66	.....	.....	.....	.....

control group (set E) showed no such correlation.

IV. PHENOTYPIC ABNORMALITIES

The only morphological abnormality that occurred more frequently in treated than in control animals was one affecting the abdomen. It resembles somewhat the mutant abnormal abdomen but is more like the highly variable, peculiarly inherited abnormality reported by Morgan (1915) and Green (1940), in that the segments are distorted, spiraling, or fragmentary. The ether-induced abnormality was not inheritable. Rapoport (1939) has produced uninheritable abnormality of the abdomen by feeding selenium or colchicine.

No instance of the abdominal abnormality was found in the controls, and only 6 in 1,000 flies from the stock. Abnormalities were distributed as follows: set A, 16 (15.5 per cent of the flies emerged); set B, 4 (9.5 per cent); set C, 3 (2.3 per cent); and set D, 1 (1.7 per cent). Abdominal abnormality, like mortality, appears to decrease with increase of age at etherization, but more data for sets B and D are needed to verify this. This age relationship will receive further attention, since production of abnormal adults by treating eggs less than 7 hours old is not in agreement with Geigy's generalization (1931) that imaginal defects are produced only when embryos older than 7 hours are irradiated with ultraviolet.

Abnormality resembles mortality also in its relation to developmental rate. In set A, 21.1 per cent of the flies pupating later than 1 σ beyond the mean had abnormal abdomens, as against 13.3 per cent in those pupating within 1 σ of the mean, and none in those earlier than 1 σ from the mean. Distribution at emergence was 40.0 per cent in the late, 13.2 per cent in the middle, and none in the early.

None of the emerged flies showed polyploid features (coarser wing and eye

TABLE 2

DELAY INCURRED DURING EACH OF THE THREE FIRST DEVELOPMENTAL STAGES

Delay is expressed as percentage of the normal duration of the embryonic, larval, or pupal stage.

Set	Embryonic	Larval	Pupal
A.....	5.87	8.06	0.64
B.....	7.19	6.07	2.98
C.....	4.76	5.41	−0.22
D.....	2.68	3.71	0.40

TABLE 3

CORRELATION OF MORTALITY AND DELAY

The pupation time is divided into three areas of the distribution curve. The central area, extending 1 σ on each side of the mean ($M \pm 1\sigma$) comprises those flies which pupated nearest the average time, as against the more advanced ($< 1\sigma$) and more retarded ($> 1\sigma$) pupation times. The percentage of mortality within each such group represents the proportion of flies which did not emerge from the timed pupae.

Set	Less than 1 σ		Mean $\pm$ 1 σ		More than 1 σ	
	No. of Pupae	Percentage of Mortality	No. of Pupae	Percentage of Mortality	No. of Pupae	Percentage of Mortality
A.....	3	0	90	4.4	19	21.1
C.....	5	0	119	2.5	13	15.4
E.....	3	0	184	1.1	28	00.0

structure, blunter body). To check on gross examination the imagos derived from treated eggs were crossed with untreated wild-type flies. Records were kept of their fertility, and their offspring were examined for polyploid features. No indisputable instance of a polyploid adult could be obtained.

Braungart and Ott (1942) were able to obtain polyploid tissues in *Drosophila* larvae with colchicine, but they limited their studies to immediate effects. Similar experiments by Cornman (unpublished), in which colchicine was administered by immersing eggs or injecting larvae, produced increased mortality but no polyploid adults. It must be concluded that, while polyploidy can be established in an embryo or in a larva, the individual rarely, if ever, survives to the imaginal stage. Unlike plants, animals as a rule show this refractoriness in maintaining polyploidy.

DISCUSSION

I. FACTORS CAUSING DELAY IN DEVELOPMENT

Delay can result from a simple slowing of metabolic processes by the ether or from disturbances caused by ether injury, or both. Delay in hatching could be considered a simple retardation in set B as against set A, corresponding to the greater visible activity in set B at the time of etherization. The relationship is reversed between sets C and D, however, with set D etherized during the time of greater activity but showing the lesser delay. Moreover, in all sets the average larval stage is also prolonged, although there was no exposure to ether during that period. Such an after-effect is not to be expected where the experimental agent slows development without causing injury. Powsner (1935) has demonstrated that cold is such an agent, producing extreme delay in the development of *Drosophila*, and only during the exposure.[2] The delay produced by ether, then, must be attributed to at least two factors: the readily reversible narcotization and the less easily repaired morphological alterations.

In this connection the pupation data for set A are suggestive in that the considerable delay but low mortality might be interpreted as an indication of considerable regulation which slowly eliminated the ether-induced abnormalities.

II. CORRELATION OF EFFECTS

It is interesting that the percentage of eggs with ether-induced cytological abnormalities is about equal to the ether-induced mortality, but too much weight should not be given to these figures because all cytological and phenotypic abnormalities cannot be detected. Nor does this merely quantitative correlation prove that these individuals with well-defined cytological abnormalities were the ones that died during the first three stages of the life-cycle.

Correlation may well be expected between phenotypic abnormality and delay in development. The distribution of abnormality in set A confirms this. The late-pupating flies show one and one-half times the incidence of abnormality found in the middle group; the late-emerging flies, three times the abnormality of the middle group. Mortality, as well, is higher among delayed individuals. Data are not available for the distribution of mortality in the larval stage, since raising larvae separately increases

[2] Powsner does obtain a slight secondary delay with lower temperature: larval period at 18°, and pupal period and control at 25°. This he attributes to a difference in the temperature coefficient of puparium formation and developmental processes in general. He does not consider the possibility that the very prolonged period may have produced abnormalities. Data on the mortality might have indicated the presence of injuries.

mortality. However, we are able to compare the mortality of flies retarded at pupation with those not retarded (Table 3). The mortality of the delayed individuals is relatively high, although the low mortality for the slower members of the control (set E) shows that delay does not necessarily reflect a moribund condition. These comparisons are also consistent with the idea that abnormalities, retardation, and death have, to a certain extent, common causes.

The high mortality of delayed individuals also supplies an explanation for the decrease in relative delay as the flies develop. Between pupation and emergence the slowing of development is almost negligible (Table 2). It appears that the retarded individuals eliminated during the pupal period allow the average rate of development to return almost to normal. This does not mean that delay is caused only by abnormalities of the type that lead to death. Rather, the variance between mortality and delay in Table 1 indicates that additional factors are upset which find expression only in retarded development.

III. ETHER AS AN ANESTHETIC

In view of the marked effect of ether on the chromatin of the *Drosophila* embryo, one must consider the possibility of mutations resulting from the treatment. There are increasing numbers of reports of mutations produced by chemicals, including narcotics; and there is also the resemblance of cytological ether effects to those of radiations and heat, which are known to produce mutations. Nevertheless, numerous experiments have shown no influence of ether on the mutation rate of *Drosophila* (Morgan, 1914; Hanson and Heys, 1933; Kossikov, 1935). Nor was the ether-induced abdominal abnormality described here inherited. Possibly a dosage as heavy as that used in these experiments would produce a genetic effect. The flies tested by Kossikov and by Hanson and Heys were merely anesthetized, whereas a 20-minute exposure to an atmosphere one-third ether is near the limit of viability. Of 140 adults so etherized, 15 showed signs of life after 1 hour, and 4 eventually recovered enough to walk. A ClB analysis of the offspring of heavily or repeatedly etherized flies would give a fairer estimate of the potency of ether as an inducer of mutations.

However, Hanson and Heys did report that, while ether alone had no significant effect, it increased the production of lethal mutations and sterility in X-rayed *D. melanogaster*. Kossikov, on the contrary, obtained no augmented rate of X-ray-induced lethal mutation in anesthetized *D. simulans*. Accordingly, while particular care might be advisable where a combined effect could be involved, the difference between the dosage employed in experiments presented here and the usual anesthetizing dose precludes any equivalent effects in routine genetics experiments.

SUMMARY

1. *Drosophila* eggs exposed to ether at different embryological stages showed abnormalities in cytology and development.

2. The chromatin became fused, producing pycnotic nuclei and agglutinated chromosomes. Some nuclei and spindles acquired unusually large amounts of chromatin. Spindles showed many grades of multipolarity and degeneration.

3. The yolk globules took on a variety of shapes not found in normal eggs. The cytoplasm formed islands of abnormal size and became vacuolated.

4. The many harmonious processes of the egg were disorganized. Nuclei did not divide synchronously or migrate to

the periphery at the normal time. In older eggs, cells gathered in undifferentiated masses instead of forming embryonic structures.

5. Such visible abnormalities increased in severity with increase in the time between etherization and fixation, and decreased with increase in age at which eggs were etherized.

6. There was mortality increase and delay in all developmental stages.

7. Adults showed abnormality of the abdomen.

8. These cytological and developmental phenomena show definite correlation and give some insight into the mechanism of development, but the factors involved are too complex to permit arranging them in any causal sequence as yet.

9. The dose used was about 90 per cent lethal to adult flies; and therefore such marked effects are not to be expected as a result of routine anesthetization, but the possibility of a synergistic effect should be guarded against.

LITERATURE CITED

BRAUNGART, D. C., and OTT, G. E. 1942. A cytological study of the effect of colchicine on *Drosophila melanogaster*. Jour. Hered., **33**:163–65.

GEIGY, R. 1931. Erzeugung rein imaginaler Defekte durch ultraviolette Eibestrahlung bei *Drosophila melanogaster*. Arch. f. Entw'mech., **125**:406–47.

GREEN, W. R. 1940. The significance for the theory of heredity of the reduction in the number of body segments in *Drosophila melanogaster* through rigorous selection. Jour. Morph., **66**:67–92.

HANSON, F. B., and HEYS, F. 1933. The relation of the induced mutation rate to different physiological states in *Drosophila melanogaster*. II. Irradiation during complete anesthesia. Amer. Nat., **67**:419–28.

HARNLY, M. H. 1929. An experimental study of environmental factors in selection and population. Jour. Exper. Zoöl., **53**:141–70.

HOCQUETTE, M., and VILLARD, R. 1932. Action de l'éther en vapeurs saturantes sur les noyeaux quiescents et en cinèse des plantules de *Raphanus sativus*. Compt. rend. Acad. sci., **195**:262–66.

HUETTNER, A. F. 1923. The origin of the germ cells in *Drosophila melanogaster*. Jour. Morph., **37**: 385–423.

———. 1924. Maturation and fertilization in *Drosophila melanogaster*. Jour. Morph. and Physiol., **39**:249–65.

———. 1927. Irregularities in the early development of *Drosophila melanogaster*. Zeitschr. f. Zellforsch. u. mikr. Anat., **4**:599–610.

———. 1933. Continuity of the centrioles in *Drosophila melanogaster*. *Ibid.*, **19**:119–34.

KOSSIKOV, K. V. 1935. The lack of effect of etherization on the X-ray mutation rate in *Drosophila simulans*. Genetics, **20**:363–65.

MORGAN, T. H. 1914. The failure of ether to produce mutations in *Drosophila*. Amer. Nat., **48**:705–11.

———. 1915. The role of the environment in the realization of a sex-linked Mendelian character in *Drosophila*. *Ibid.*, **49**:385–429.

PINCUS, G., and WADDINGTON, C. H. 1939. The effects of mitosis-inhibiting treatments on normally fertilized pre-cleavage rabbit eggs. The comparative behavior of mammalian eggs *in vivo* and *in vitro*. V. Jour. Hered., **30**:515–18.

POLITZER, G. 1934. Pathologie der Mitose. "Protoplasma Monographien," No. 7. Berlin: Gebrüder Borntraeger.

POULSON, D. F. 1937. The embryonic development of *Drosophila melanogaster*. Actualités sci. et ind. Exposés de génétique, III. Paris: Hermann & Cie.

POWSNER, L. 1935. The effects of temperature on the durations of the developmental stages of *Drosophila melanogaster*. Physiol. Zoöl., **8**:474–520.

RABINOWITZ, M. 1941. Studies on the cytology and early embryology of the egg of *Drosophila melanogaster*. Jour. Morph., **69**:1–43.

RAPOPORT, J. A. 1939. Specific morphoses in *Drosophila* induced by chemical compounds. Bull. de biol. et de méd. expér. de l'URSS, **7**:415–17.

RICHARDS, A. G., JR., and MILLER, A. 1937. Insect development analyzed by experimental methods: a review. Jour. New York Entomol. Soc., **45**: 1–60.

ROSENFELD, M. 1932. The action of ether on cells in mitosis. Arch. f. exper. Zellforsch., **12**:570–86.

SONNENBLICK, B. P. 1940. Cytology and development of the embryos of X-rayed adult *Drosophila melanogaster*. Proc. Nat. Acad. Sci. (U.S.A.), **26**:373–81.

WILSON, E. B. 1901. Experimental studies in cytology. II. Arch. f. Entw'mech., **13**:353–95.

PLATE I

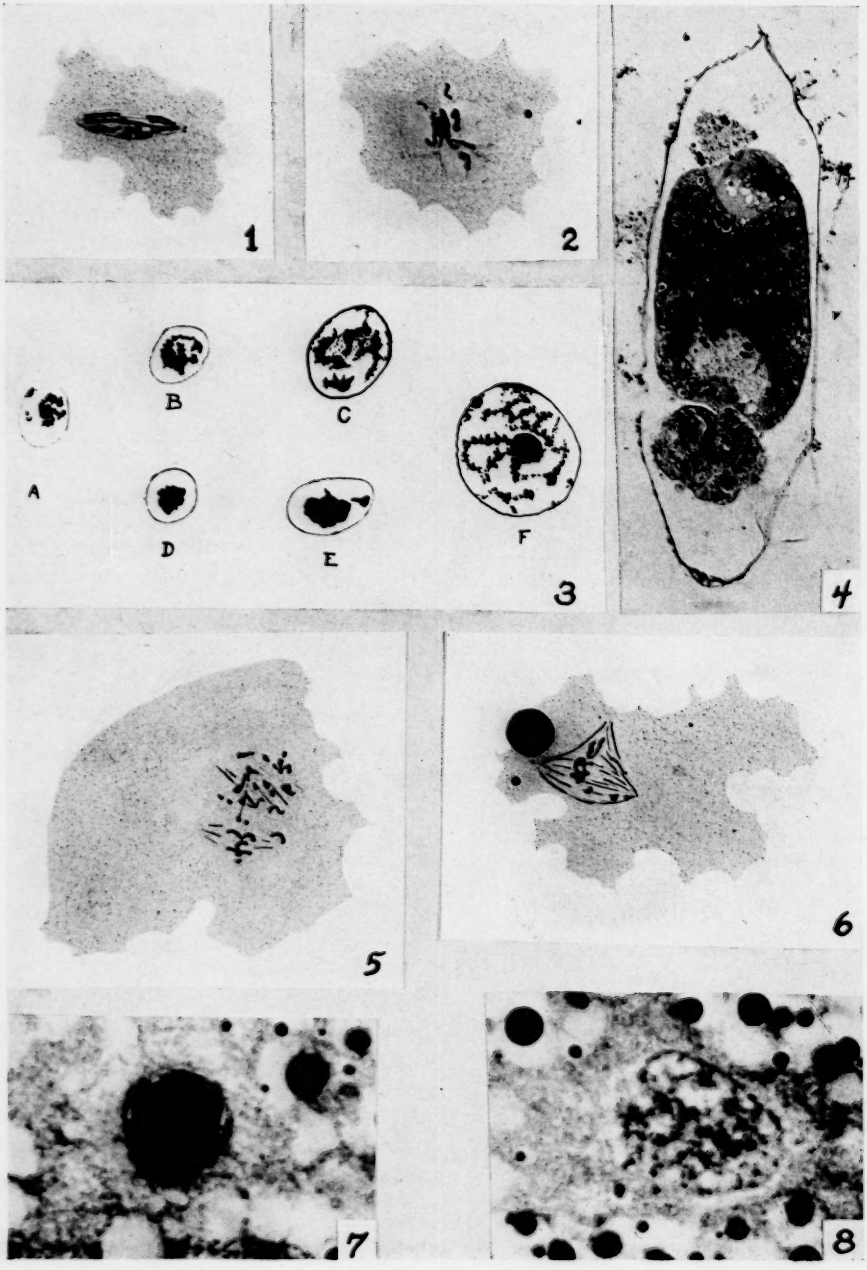

PLATE I

All the eggs were from set A, etherized when 5–20 minutes old. The recovery period (time between end of exposure and fixation) is indicated for each figure. All eggs included here were fixed in formol-alcohol-acetic fixative and stained with Heidenhain's iron haematoxylin. The magnification for Fig. 4 is 160 ×; for the others, 1,300 ×. Figs. 1, 2, 3, 5, and 6 are drawings.

FIG. 1.—Chromatin bridge resulting from the inability of chromosomes to separate at anaphase. In the same egg there were clumped chromosomes and pycnotic nuclei. Recovery time, 40 minutes.

FIG. 2.—Clumping of chromosomes and separation of chromosomes from the spindle remnant. Recovery, 60 minutes.

FIG. 3.—Granular (*B*, *C*, *F*) and pycnotic (*D*, *E*) nuclei showing the enormous differences in size and chromatin content that can occur within one egg. A degenerating yolk nucleus from a normal egg is included for comparison (*A*). Recovery, 90 minutes.

FIG. 4.—Photograph of a frontal section through an egg and its membranes. Nuclei are scattered throughout the cytoplasm. Yolk and cytoplasm are separating irregularly. Development has proceeded without organization. Recovery, 35 hours (15 hours after normal hatching time).

FIG. 5.—Multipolar spindle with irregularly distributed chromosomes. Vacuoles surround the periphery of the spindle. The faintness of the spindle fibers suggests that the spindle has already begun to dissolve, before anaphase separation has occurred, so that a single giant nucleus would have resulted. A giant nucleus was present in the same egg. Recovery, 3 hours 20 minutes.

FIG. 6.—Tripolar spindle with chromosomes apparently being drawn toward all poles. Several multipolar spindles were found in this egg. Recovery, 3 hours 20 minutes.

FIGS. 7 AND 8.—Photographs of two giant nuclei. The one has an almost normal reticular appearance, while the other contains a dense chromatin mass. No other nuclei were found in this egg. Recovery, 90 minutes.

THE DEVELOPMENT OF REACTIVITY IN THE ACCESSORY REPRODUCTIVE ORGANS OF CASTRATED AND SPAYED RATS INJECTED WITH TESTOSTERONE PROPIONATE[1]

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IT HAS been established that the qualitative and quantitative responses of the gonads and accessory reproductive organs to exogenous hormones depend not only upon the hormones administered, the length of treatment, and the dosage employed but also upon the age of the treated animals. The importance of age as a factor in the induction of precocious maturity of the reproductive tract of the male and female rat and mouse was clearly demonstrated by early work of Smith (1926, 1927) and Smith and Engle (1927*a*, *b*). Hooker (1942) reported that there was a marked increase in sensitivity to testosterone treatment in the seminal vesicles of castrated rats at the age of puberty (40–60 days) but that this sensitivity was lost in older animals. Price and Ortiz (1944) and Price (1944) treated male and female rats at various ages with equine

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gonadotropin, testosterone propionate, and estradiol benzoate. It was found that the initial responses to stimulation of the gonads and accessory reproductive organs, as determined by histological changes and percentage-weight increases over the normal, were small. Reactivity increased with age, and a peak in percentage-weight increase was reached at 26 days of age for the majority of the organs studied, although the testis and male and female prostate glands gave a maximum response at 14 or 18 days. There was a decline in responsiveness after 26 days of age in all organs examined except the female prostate gland. The fact that there was no decline in reactivity in the female prostate, which normally regresses in weight after 36 days of age, and that the drop in percentage-weight increase in all the other organs occurred at ages when these organs in control animals were beginning to grow very rapidly suggested that the loss in responsiveness was only apparent. It appeared that, when the rapid growth associated with puberal changes had begun, the organs were no longer able to increase to the same degree in percentage weight above the normal. To clarify the problem of the development and maintenance of responsiveness it seemed advisable to remove the gonads and determine the reactivity of the accessory reproductive organs to exogenous hormones without the complicating factor of endogenous, gonad-secreted hormones. The results of such a study are reported here.

MATERIAL AND METHODS

Albino rats of a selected inbred stock which has been developed in this laboratory were used. This stock has a high percentage of female prostate glands. Two types of experiments were performed: in one, males and females were gonadectomized at 8 days of age and were daily injected subcutaneously with graded doses of testosterone propionate for 6-day periods, beginning at 8, 20, 30, 50, or 70 days of age. Twenty-four hours after the last treatment was given, the animals were autopsied. It was recognized that the length of time which elapsed between gonadectomy and treatment might influence reactivity. To rule out such a factor a second series of experiments was performed in which males were castrated at 20, 30, 50, or 70 days and injected immediately for the standard 6-day period as in the first series of experiments. The study of the second series is incomplete, but some of the crucial data for the 76-day period will be presented here.

At autopsy the prostates and seminal vesicles of the males and the prostates and uteri of the females were dissected with the aid of a wide-field dissecting microscope, weighed on a torsion balance, and fixed in Bouin's fluid for histological study. Samples of the male prostates and seminal vesicles were sectioned at 7 μ thickness, and the female prostates and portions of the uteri were cut serially at 10 μ. Prostates and uteri were stained in Harris' haematoxylin and the seminal vesicles in Ehrlich's haematoxylin.

Two hundred and sixty-six males and females were used in the first series of experiments, and 100 males in the second series (which will be reported only briefly and in part). Female prostate glands were recovered from 61 controls and 73 treated females. Tissues from 134 animals were studied histologically.

It was desirable to determine the lowest effective dose of testosterone propionate to which the organs could respond at the various ages and to find the maximum response which could be given to relatively high doses. The dosages

selected for daily administration were 5, 10, 25, 50, and 100 μg. (the standard dose given in earlier studies). The criteria used for determining the reactivity of the organs were the percentage increase in weight above the normal control and definite histological changes.

The weight data were treated as reported earlier (Price and Ortiz, 1944). That is, the percentage change in weight of organs of treated animals as compared with that of littermate controls was determined for each litter, and the mean percentage change in weight for organs in all litters similarly treated at each age was obtained. These mean percentage changes are given in Table 2. Female prostate glands were not found in all females; and in cases where the controls lacked prostates the mean weight of the prostate glands found in other control animals of that age was substituted as a control weight. This was also done if the lobes (bilateral, or right or left unilateral) did not correspond in the control and treated females.

The histological changes which seemed the most dependable criteria for the effectiveness of hormone treatment were the presence of secretion granules in the seminal vesicles and light areas in the epithelium of the male and female prostate. However, the development of seminal vesicle secretion granules required so large a dose of androgen that a second criterion—epithelial height—was used to detect the effects of smaller doses, even though the changes were more difficult to evaluate. The epithelium of the prostate is more sensitive to small doses of male hormone than that of the seminal vesicles. However, the use of this gland in determining the effectiveness of small doses of hormone by histological criteria is limited because of the fact that the prostates of castrated males and spayed females attain a differentiated state histologically for a period of time. This is not a serious difficulty, since the histological state of the gland can be used at 14, 56, and 76 days of age as a definite indicator (that is, before the development of light areas in the prostates of gonadectomized controls and after light-area regression).

In the second series of experiments in which castrations were performed at such ages as 56 and 76 days, after the histological differentiation of both prostates and seminal vesicles had occurred, the criteria of effectiveness and reactivity were maintenance of organ weights in treated males and maintenance of histological differentiation. This involved the preservation of light areas in the prostate and secretion granules or moderately high epithelium in the seminal vesicles. Castration at these ages results in loss of secretion granules in 24 hours, of light areas within 4 days, and a rapid loss in organ weights. Table 3 shows the results of treatment of litters immediately after operation at the youngest and oldest ages.

The only criterion for reactivity in the uterus was weight change, since none of the doses used caused any differentiation of the endometrium. Growth in both endometrium and myometrium occurred as a result of treatment.

Table 1 summarizes the effects of graded doses of androgen upon mean organ weights at the various ages and shows the effective doses as judged by histological criteria. The mean weights for female prostates include only cases with bilateral lobes. Data for unilateral lobes were included in the computation of percentage-weight increases in Table 2. In spite of the small number of cases in each category and the variability in weight of the male and female prostate in gonadectomized animals, the trends of weight change are clear. A larger num-

TABLE 1

SUMMARY OF THE RESPONSES OF ORGANS TO TESTOSTERONE PROPIONATE TREATMENT

Sex	Autopsy Age (Days)	Castrate Control			Testosterone Propionate Treated Rats*																				
					100 μg.			50 μg.			25 μg.			10 μg.			5 μg.			1 μg.					
		No.	M. Wt. Mg.†		No.	M. Wt. Mg.		No.	M. Wt. Mg.		No.	M. Wt. Mg.		No.	M. Wt. Mg.		No.	M. Wt. Mg.		No.	M. Wt. Mg.				
			♂Pr.	S.V.		♂Pr.	S.V.		♂Pr.	S.V.		♂Pr.	S.V.		♂Pr.	S.V.		♂Pr.	S.V.		♂Pr.	S.V.				
♂ ♂	14	9	2.2	2.1	4	+‡ 8.8	+ 13.9	2	+ 9.9	+ 11.2	2	+ 9.8	+ 11.3	3	+ 6.5	7.8	3	+ 6.2	6.9	3	+ 3.3	3.6			
	26	6	+ 11.0	2.6	3	+ 30.6	+ 27.0	3	+ 26.4	+ 23.3	3	+ 26.6	+ 21.0	3	+ 15.0	9.2	3	+ 14.5	5.7	3	+ 11.3	2.7			
	36	5	6.0	2.6	2	+ 43.8	+ 29.6	2	+ 45.6	+ 26.2	2	+ 31.8	+ 21.7	3	+ 19.8	8.0	3	+ 13.0	4.2	2	+ 6.8	3.1			
	56	4	4.6	2.6	2	+ 45.8	+ 35.1	1	+ 41.2	+ 36.4	2	+ 23.4	+ 20.2	4	+ 9.9	7.1	3	+ 6.5	5.9	2	4.9	3.2			
	76	10	5.1	3.8	2	+ 45.5	+ 36.3	1	+ 36 0	+ 35.0	3	+ 24.0	+ 19.4	2	+ 11.7	10 2	2	6.6	4.3	1	6.8	3.8			
			♀Pr.	Ut.		♀Pr.	Ut.		♀Pr.	Ut.		♀Pr.	Ut.		♀Pr.	Ut.		♀Pr.	Ut.		♀Pr.	Ut.			
♀ ♀	14	13 (9)§	0.8	12.0	5 (2)	+ 4.6	17.4	2 (2)	+ 3.2	13.3	4 (3)	+ 3.3	15.2	2 (2)	+ 2.4	11.0	5 (1)	+ 2.0	12.0	5 (1)	+ 1.4	11.4			
	26	17 (11)	+ 5.6	15.4	4 (3)	+ 23.6	66.0	2 (2)	+ 13.0	52.4	2 (2)	+ 13.2	38.5	4 (1)	+ 9.2	16.2	4 (1)	+ 3.8	15.9	2 (2)	5.1	15.1			
	36	14 (6)	3.7	16.2	4 (3)	+ 24.0	73.0	5 (3)	+ 17.5	48.2	4 (3)	+ 10.8	32.7	4 (2)	+ 4.5	17.5	5 (2)	4.1	17.0	2 (1)	4.4	14.5			
	56	12 (4)	2.6	20.0	4 (2)	+ 18.4	77.7	3 (1)	+ 12.8	49.9	2 (1)	+ 6.4	35.3	2 (2)	+ 4.0	20.9	3 (1)	4.8	23.5	2 (1)	2.6	20.0			
	76	7 (7)	1.7	23.3	3 (2)	+ 21.1	75.3	3 (1)	+ 12.0	42.0	3 (2)	+ 8.7	29.6	2 (1)	+ 3.4	24.1	2 (2)	+? 1.9	22.0	1 (1)	2.0	23.8			

* Gonadectomized at 8 days; treated for a 6-day period prior to autopsy.

† M. Wt. Mg. = mean weight in milligrams; Pr. = prostate; S.V. = seminal vesicles; Ut. = uterus.

‡ Plus signs (+) indicate the presence of light areas in the prostate or secretion granules in the seminal vesicles.

§ Figures in parentheses indicate the number of females with prostate glands.

TABLE 2

MEAN PERCENTAGE CHANGES IN WEIGHT OF ORGANS WITH ANDROGENIC TREATMENT*

Autopsy Age (Days)	Daily Dose of Testosterone Propionate																							
	100 μg.				50 μg.				25 μg.				10 μg.				5 μg.				1 μg.			
	S.V.	♂Pr.	♀Pr.	Ut.	S.V.	♂Pr.	♀Pr.	Ut.	S.V.	♂Pr.	♀Pr.	Ut.	S.V.	♂Pr.	♀Pr.	Ut.	S.V.	♂Pr.	♀Pr.	Ut.	S.V.	♂Pr.	♂Pr.	Ut.
14....	570	283	415	61	490	351	300	45	495	345	276	33	263	187	200	− 2	163	153	100	4	38	35	75	3
26....	1,237	249	454	326	1,057	201	176	279	949	188	191	167	281	40	80	7	139	33	20	1	9	10	−45	−3
36....	1,380	879	556	335	1,010	872	496	203	985	602	180	103	203	216	69	12	57	117	47	10	5	− 2	19	−0.5
56....	1,198	871	739	269	1,200	664	654	176	675	394	190	143	171	105	53	11	128	41	41	13	23	12	17	12
76....	967	917	1,282	242	955	650	606	84	593	456	411	32	193	157	100	9	10	28	8	0	0	13	17	7

* Males and females were gonadectomized at 8 days of age and treated with androgen for 6 days prior to autopsy.

ber of cases would undoubtedly have eliminated some of the apparent discrepancies.

EXPERIMENTAL RESULTS

As discussed above, this study of the reactivity of organs to testosterone propionate treatment involved two separate phases; that is, the determination of the minimum effective dose at the various ages, as judged by histological criteria and weight changes, and the determination of the maximum responses based upon the percentage-weight increase with high doses.

MALE PROSTATE

At 14 days of age the minimum effective dose for both weight increase and histological change was 1 μg. daily. The mean weights in Table 1 and the mean percentage changes in weight in Table 2 show clearly that the gland was sensitive to this dose. The mean weight of 3.3 mg. (range, 3.2–3.4) in animals treated with 1 μg. daily was well above the mean weight of 2.2 mg. (range 2.0–2.6 mg.) in the glands of castrated controls. The histological findings proved conclusively the responsiveness of the prostate to 1 μg. In the prostates of castrated controls at 14 days of age (Pl. I, Fig. 1) the acini were small, and many were still solid. Treatment with 1 μg. of androgen daily caused enlargement of the acini, the formation of lumina, increase in epithelial height, and the development of pale light areas in the cells of some acini (Fig. 2). The presence of tall columnar epithelium which contains light areas is considered a criterion of a "functional" adult type of gland. Dosages of 5 μg. and above resulted in markedly better histological development than that produced by 1 μg., which appeared to be approximately the lowest dose which would have caused light-area development.

By 26 days of age the prostate gland of castrated controls had increased markedly in weight and had differentiated so that distended acini, tall columnar epithelium, and light areas were present, as reported by Price (1936). Because of this differentiation, no determination of the lowest effective dose on the basis of histological change could be made. Androgen treatment in this period of normally rapid growth of the gland was less effective, as judged by percentage change in weights above the control prostates, than at 14 days of age. The lowest effective dose was 25 μg. (The increases with 10, 5, and 1 μg. cannot be considered significant because of the small number of cases and the fact that the weights in treated rats fell within the range of control weights.)

In the 36-day-old group the castrate control glands had regressed, as shown by weight reduction and histological changes. No light areas were found in the low prostate epithelium, and the acini were small. The injection of 1 μg. of androgen apparently caused the maintenance or development of light areas, but it could not be concluded that this was the minimum effective dose because light areas have sometimes been found in castrate controls at this age. From weight changes it was determined that the minimum effective dose was 5 μg.

In the 56-day-old group, histological criteria of effectiveness could again be used with certainty, and the lowest effective dose was 5 μg. The weight increase with 5 μg. was of doubtful significance.

At 76 days a loss in reactivity was found. On the basis of histological criteria, 5 μg. was no longer effective, nor was there any significant weight increase with this dose. The lowest effec-

tive dose for histological development and weight increase at 76 days was 10 μg.

From these data a number of conclusions can be drawn. The prostate in animals treated from 8 to 13 days was very sensitive to testosterone propionate and responded in weight and in histological development to even 1 μg. daily. As the prostate grew and differentiated, the minimum effective dose on the basis of weight became, at 26 days, 25 μg., presumably because in a rapid growth phase all doses were somewhat less effective and low doses were especially so. At 36 and 56 days of age after gland regression, the minimum effective dose, from weights and histology, was 5 μg.; at 76 days it was 10 μg. These rather complicated data indicate that, on the basis of evidence from minimum effective doses for weight changes and histology, there was a loss in reactivity at 26 days, an increase at 36 days (but not attaining the level of sensitivity at 14 days), and a progressive loss thereafter.

A study of the responses in weight at the various ages with maximum doses did not support the idea of loss in reactivity at the most advanced ages. On the basis of evidence from the percentage-weight increases, there was a slight loss, as compared with 14 days, at 26 days (coinciding with rapid growth), but by 36 days of age there was a marked increase in reactivity. Percentage-weight increases of approximately the same magnitude were maintained, at least with 100 μg., at 36, 56, and 76 days of age.

To determine whether the loss in reactivity as shown by minimum-dose requirements at 56 and 76 days was an actual one, studies were made of litters castrated at 70 days and injected immediately for the standard period with the graded doses of androgen. Table 3 gives the results, and, for comparison, litters treated at 14 days are included. As shown in litters 3 and 4, castration at 70 days of age caused immediate loss in prostate weight and in differentiation. The prostate shown in Figure 5 was the better developed of the two castrated controls; and it can be seen that, although the acini are still moderately distended, the epithelium is low and of a typically castrate type. The injection of 5 and even 1 μg. of androgen partially prevented loss in prostate weight, and, as would be expected, larger doses were more effective. Even more striking is the fact that injection of 1 μg. daily prevented prostate involution and maintained larger acini, moderately high epithelium, and pale light areas (Fig. 6). The effects of 5 μg. were markedly greater, and well-developed light areas were maintained in tall columnar epithelium.

It is apparent that if androgen was administered immediately after castration in the youngest and oldest age groups, 1 μg. produced definite effects on organ weights and histology. Light areas were produced at 14 days, several days before they would normally have appeared, and were maintained at 76 days when prostate involution had occurred in castrate controls. It is quite possible that slight detectable effects on acinous distention might have been obtained with even lower doses than 1 μg., but it seemed desirable to use a sharp, detectable criterion, such as the development or maintenance of light areas.

SEMINAL VESICLES

A study of the seminal vesicles provides more easily interpretable results because these glands do not differentiate after castration and their growth is slight. The epithelium remains low and the structure relatively simple. How-

ever, they were not so sensitive to androgen, at least so far as the development of definite secretion granules was concerned, and other histological criteria were more difficult to evaluate. tive dose for causing a slight increase in epithelial height was 5 μg., but a minimum of 25 μg. was necessary for the development of secretion granules at this and all subsequent ages.

TABLE 3

EFFECTS OF ADMINISTRATION OF TESTOSTERONE PROPIONATE IMMEDIATELY AFTER CASTRATION

Litter No.	Autopsy Age (Days)		Body Wt. (Gm.)	Prostate		Seminal Vesicle	
				Wt. (Mg.)	Histology*	Wt. (Mg.)	Histology*
1.....	14	Cast. at 8 days; inj. 8–13 with 100 μg. t.p. daily	19	6.8	Distended acini; high epithelium with l.a.	12.8	High epithelium; sec. grs.
		Cast. at 8 days; inj. 8–13 with 50 μg. t.p. daily	21	9.8	Distended acini; high epithelium with l.a.	11.6	High epithelium; sec. grs.
		Cast. at 8 days; inj. 8–13 with 25 μg. t.p. daily	20	8.0	Distended acini; high epithelium with l.a.	11.8	High epithelium; sec. grs.
		Cast. at 8 days; cast. control	19	2.2	Most acini small and solid	2.0	Low epithelium
		Normal control	21	4.8	Moderately distended acini; high epithelium with l.a.	3.6	Low epithelium
2.....	14	Cast. at 8 days; inj. 8–13 with 5 μg. t.p. daily	21	5.8	Distended acini; high epithelium with l.a.	6.4	Low epithelium; some slightly higher than cast. control
		Cast. at 8 days; inj. 8–13 with 1 μg. t.p. daily	24	3.4	Most acini solid; some with high epithelium and pale l.a. (Fig. 2)	3.6	Low epithelium
		Cast. at 8 days; cast. control	23	2.4	Most acini small and solid; some with lumina (Fig. 1)	2.6	Low epithelium
		Normal control	24	4.8	Moderately distended acini; high epithelium with l.a.	4.0	Low epithelium
3.....	76	Cast. at 70 days; inj. 70–75 with 100 μg. t.p. daily	120	172.6		660.8	
		Cast. at 70 days; inj. 70–75 with 50 μg. t.p. daily	147	167.8		353.6	
		Cast. at 70 days; inj. 70–75 with 25 μg. t.p. daily	146	106.0		244.0	
		Cast. at 70 days; inj. 70–75 with 10 μg. t.p. daily	132	90.0	Distended acini; high epithelium with l.a.	128.0	Low to moderate epithelium
		Cast. at 70 days; inj. 70–75 with 5 μg. t.p. daily	131	78.4	Distended acini; high epithelium with l.a.	70.2	Low epithelium; some slightly higher than cast. control
		Cast. at 70 days; inj. 70–75 with 1 μg. t.p. daily	131	60.4		104.0	
		Cast. at 70 days	148	43.8	Moderately distended acini; low epithelium; no l.a. (Fig. 5)	75.0	Low epithelium
		Cast. control					
		Normal control	160	178.6	Distended acini; high epithelium with l.a.	387.4	High epithelium; sec. grs.
4.....	76	Cast. at 70 days; inj. 70–75 with 5 μg. t.p. daily	214	109.0	Distended acini; high epithelium with l.a.	120.4	Low epithelium; some slightly higher than cast. control
		Cast. at 70 days; inj. 70–75 with 1 μg. t.p. daily	180	85.0	Distended acini; moderately high epithelium with pale l.a. (Fig. 6)	113.8	Low epithelium
		Cast. at 70 days	240	57.6	Small acini; low epithelium; no l.a.	126.8	Low epithelium
		Cast. control					

* L.a. = epithelial light areas; sec. grs. = secretion granules.

At 14 days of age the minimum effective dose was 1 μg., as determined by changes in weight. The gland weights in treated animals were definitely above the range of control weights. Mean absolute weights were 3.6 mg. (range 3.4–3.8) in treated rats and 2.1 mg. (range 1.8–2.6) in controls. The minimum effec-

At 26 days, 5 μg. was the minimum effective dose, as judged by weight changes and histological criteria; and it was still the minimum effective dose, as determined by weights, at 36 and 56 days. At 76 days of age, 10 μg. was the minimum effective dose for the production of weight changes. No definite effects

on the seminal vesicle epithelium were found after 26 days with less than 25 μg., although, in some cases, there were indications that 10 μg. might have been slightly effective.

As in the case of the male prostate, the seminal vesicles seemed to lose some of their ability to respond to small doses of androgen with advancing age. At 14 days of age the minimum effective dose from weight data was 1 μg.; at 26, 36, and 56 days it was 5 μg.; and at 76 days of age it was 10 μg. From histological criteria the minimum effective dose was 5 μg. at 14 days; but by 76 days of age it was probably 25 μg. or a little less.

A study of the response in weight to maximum doses at various ages established that there was no progressive loss in reactivity with advancing age. On the contrary, a striking increase in percentage change in weight occurred at 26 days, as compared with the 14-day period. Percentage changes of the same magnitude were found at 36 and 56 days of age; but at 76 days of age reactivity seemed to be somewhat reduced.

Since loss in responsiveness (as shown by the minimum-dose requirements) was progressively greater as the period between castration and treatment increased, it was necessary to check the results of treatment administered immediately after castration at an advanced age. Examination of the data from the two litters shown in Table 3 reveals that responsiveness was not lost when such an experimental procedure was followed. Removal of the gonads caused rapid and large losses in seminal vesicle weights in the 6-day period and a profound regression in histological condition. The secretion granules were lost, and the epithelium became very low. Injection of 5 μg. daily, given immediately after gonadectomy at 70 days, maintained a higher epithelium than in the castrate control. This was the minimum effective dose for the production of increase in height of the epithelium at 14 days. No loss in reactivity is apparent in these findings.

FEMALE PROSTATE

At 14 days of age the minimum dose of 1 μg. caused weight increases and histological changes in the female prostate. The control glands had small acini, of which the majority were still solid (Fig. 3). The injection of 1 μg. daily resulted in marked enlargement of the acini, the development of high epithelium in some acini, and the appearance of pale light areas (Fig. 4). Larger doses produced more striking histological changes. By 26 days of age differentiation had occurred in some, but not in all, castrate control glands, and histological criteria could not be used. From weight changes it was determined that 10 μg. was the minimum effective dose.

At 36 days of age the prostates of castrate control females had regressed completely histologically. The minimum dose which caused light-area development was 10 μg.; and this was also the minimum effective dose which produced an effect on weights.

In the 56- and 76-day-old groups the minimum effective dose remained 10 μg. for both weight increase and light-area development. There was one case of doubtful significance in which pale questionable light areas were found at 76 days with a dose of 5 μg., but in two other females with the same treatment the glands were in a typically castrate state.

The female prostate showed the same apparent loss in responsiveness to small doses of androgen with increasing age that was found in the male prostate and the seminal vesicles. At 14 days the gland was sensitive to even 1 μg. of androgen

daily, as demonstrated by both weight and histological changes. By 26 days of age the lowest effective dose had risen to 10 μg., and this remained the minimum effective dose through all the subsequent ages.

The injection of maximum doses resulted in marked percentage-weight increases at 14 days. A slight reduction in reactivity appeared at 26 days (with 50 μg.), which was probably correlated with rapid growth of the gland. An increase in percentage-weight change was evident at 36 days, and changes of even greater magnitude were found at 56 and 76 days of age. This was the more striking because of the degree of weight reduction in the prostates of castrated control females at these advanced ages.

From the findings in experimental males castrated at 70 days and treated immediately with androgen it was determined that there was no loss in reactivity of the male prostate and seminal vesicle to small doses if no time elapsed between operation and treatment. It is probable that the same is true for the female prostate, although no experiments were performed to establish the fact.

UTERUS

The effect of androgenic treatment on the uterus was one of growth of all parts of the organ without differentiation of the mucosa. Increase in weight was the only criterion of effect.

Treatment at 14 days produced weight increases that were small but definite with the highest doses. The minimum effective dose was 25 μg., and that remained true at all ages. By 26 days, increase in responsiveness to the three largest doses had occurred, as shown by weight data. This responsiveness was maintained at 36 and 56 days, but by 76 days of age a loss was apparent. The uterus evidently followed the pattern of the other organs in increasing in responsiveness with maximum doses. Some loss of reactivity with advancing age occurred. The uterus was far less sensitive to testosterone treatment than the other organs, which are normally under the control of androgens of testicular or ovarian origin. The minimum effective dose of 25 μg. was much above the minimum effective doses of 1 and 5 μg. (depending upon age and time of operation and treatment) for the male and female prostate glands and the seminal vesicles. However, an intermediate dose between 10 and 25 μg. might have been effective on the uterus.

DISCUSSION

The experimental data which have been presented establish clearly a trend in reactivity to androgenic treatment which was common to the male and female prostate, the seminal vesicles, and the uterus. When doses of 25, 50, or 100 μg. of testosterone propionate were administered daily, there were responses in percentage increase in weight in all the organs at the first autopsy age of 14 days. By 26 days of age in the seminal vesicles and uterus and by 36 days in the male and female prostate gland an abrupt rise in reactivity had occurred. This responsiveness, which was shown in large percentage-weight increases, was maintained through the experimental period of 76 days in the male and female prostate; it was somewhat diminished at 76 days in the seminal vesicles and was definitely reduced at 56 and 76 days of age in the uterus.

The delayed development of great reactivity in the male and female prostate glands as compared with the seminal vesicles and uterus warrants examination. The latter organs grow very slowly after gonadectomy, as shown in

the mean weights in Table 1. The male and female prostate glands, on the other hand, grow to a much greater degree and differentiate (Price, 1936, 1939), presumably under the influence of adrenal androgens (Howard, 1937, 1938; Burrill and Greene, 1939*a*, *b*, 1940, 1941). The period 14–26 days is one in which prostate growth is relatively rapid. It was demonstrated in earlier work (Price and Ortiz, 1944; Price, 1944) that when organs entered a phase of rapid growth they were not able to respond to stimulation by increasing in percentage weight above the normal to the same degree as when they were growing slowly. It seems highly probable that, if the prostate glands were not under the influence of extra-gonadal hormones in the gonadectomized animal, the peak of responsiveness would occur at 26 instead of 36 days of age.

The next question is whether there is an increase in sensitivity to small doses associated with the increase in reactivity to larger ones. With the criteria for minimum effective doses used in this study, there was no evidence of a sudden increase in sensitivity of the prostate or seminal vesicles to small doses of testosterone propionate at any age. In fact, a loss in sensitivity appeared as age advanced. However, this seemed attributable to regressive changes that had taken place in the glands because of the lapse of time between operation and treatment. It was shown (Price, 1936) that when castrations were performed at birth, certain regressive changes become apparent in the prostate and seminal vesicles after relatively long periods of time had elapsed. These involved pyknosis of the nuclei and protoplasmic changes. It seems probable that loss in reactivity was due to this factor, especially since no loss was evident as compared with the 14-day stage, when hormone treatment was begun immediately after castration of males at 70 days of age. The minimum effective dose for the male and female prostate and the seminal vesicles at 14 days of age was 1 μg. of testosterone propionate daily.

The findings in this study are not completely in agreement with those of Hooker (1942). He castrated male rats at birth and treated them for 5 days with graded doses of testosterone of 5-80 μg. daily, beginning at 10, 20, 30, 40, 50, 60, 80, 100, and 120 days. His criteria of effectiveness were responses in the seminal vesicle epithelium, which included "folding, slight increase in height, stratification of the nuclei, and mitoses," and weights of the whole prostate and seminal vesicle complex, which was weighed as a whole with a piece of urethra. He found that, at 15 days, 30 μg. produced a detectable histological and weight response, and this was also the minimum effective dose at 25 days. By 40-60 days, epithelial responses were elicited by 5 μg., and weight responses also occurred with this dose at 40 and 50 days. At 80-85 days, 20 μg. was the minimum effective dose, and, at the subsequent ages, 30 μg. was the lowest effective dose. Hooker concluded that there was a sharp increase in sensitivity, which coincided with the age when puberty normally developed, and that this responsiveness was not maintained at more advanced ages but returned to the prepuberal level.

It is a little difficult to compare Hooker's data with those presented here because weights were not taken in a comparable way, histological criteria were not exactly the same, and the injected androgen was testosterone instead of testosterone propionate. The results of treatment with the latter in the present work showed that the sensitivity of the seminal vesicles, as determined by the

minimum effective dose for weight increase, was greater at 14 days than at any other age. There was no increase in sensitivity to low doses at the 56-day period that could be determined by any criterion.

It is well established that testosterone propionate differs from testosterone in its action on the prostate and seminal vesicles of the rat. Not only is it much more effective on both glands than testosterone, but it is especially effective on the seminal vesicles, as demonstrated by Miescher, Wettstein, and Tschopp (1936), who stated: "According to the different test methods on the rat, only by using esters of testosterone, like the propionate etc. is it possible to produce in the castrated infantile rat seminal vesicles heavier than the prostate, as is the case in the normal adult rat."

The mean weights in Table 1 show that with injections of 10 μg. or more of testosterone propionate at 14 days of age the seminal vesicle weights were greater than those of the prostate. With 1 and 5 μg., seminal vesicle weights were equal to prostate weights. The special effectiveness of testosterone propionate on the seminal vesicle of this age may account for the finding of such a low minimum effective dose as compared with Hooker's findings with testosterone. It is not clear why a special sensitivity was evident at 40–60 days in his work with testosterone but not in this study with testosterone propionate.

It is interesting and instructive to compare the reactivity to androgen in organs of normal and gonadectomized animals. In noncastrated males, treatment with 100 μg. of testosterone propionate, as reported by Price and Ortiz (1944), induced a weight increase of 114 per cent in the prostate at 14 days of age; this percentage change in weight was maintained at 26 and 36 days, but it was greatly reduced at 56 days (the final experimental age), when growth was very rapid in the normal control glands. The suggestion that the apparent reduction in responsiveness was related to rapid organ growth was substantiated when treatment was given to gonadectomized rats. The injection of 100 μg. of androgen daily produced an initial percentage-weight increase of 283 per cent, which was approximately that found at 26 days. (In both normal and castrate males prostate growth was marked between 14 and 26 days.) However, at 36 days the weight change was 879 per cent—a striking increase, which was equaled or exceeded at the subsequent ages through 76 days. Apparently, the presence of testicular androgen with its stimulating effect masked the reactivity of the prostate to exogenous hormone.

The seminal vesicle reactivity showed the same phenomenon, with initial responses at 14 days of 279 per cent in the noncastrated treated male and 570 per cent in the castrated treated animal. At 26 days the increases in noncastrated and castrated rats were 656 and 1,237 per cent, respectively; at 36 days, 545 and 1,380 per cent; and at 56 days, 165 and 967 per cent. It is apparent that in the absence of gonad-secreted hormones, with the consequent slowing of the growth rate, responsiveness is increased.

The female prostate is a more difficult gland to study because of its variability in weight, even in normal animals. However, it, too, had the same increase in reactivity when comparisons were made between data for treated nonspayed females (Price, 1944) and the treated spayed females reported here. The 14-day increase was 319 per cent in nonspayed females and 415 per cent in the spayed females; at 26 days the increases

were 197 and 545 per cent; at 36 days, 347 and 556 per cent; at 56 days, 359 and 739 per cent. It must be noted that the differences between the reactivity of the prostate in nonspayed and in spayed females is not so marked as in the other accessory reproductive glands. This may be due to the fact that the female prostate undergoes weight regression after 36 days of age in both normal and spayed females. The growth curve of the gland in spayed females resembles that for normal rats but has a lower magnitude. Under such circumstances a striking difference at more advanced ages would not be expected.

The uterus was exceptional in its responses. Removal of the ovaries did not result in increased reactivity to androgen administration. In nonspayed females treated with 100 μg. of testosterone daily the uterine response at 14 days was 75 per cent; in spayed treated females, 61 per cent. At 26 days the percentage-weight changes were 375 in the nonspayed and 326 in the spayed; at 36 days, 344 and 335, respectively; and, by 56 days, 41 and 269 per cent. There was no difference between the reactivity to androgenic treatment of the uterus in nonspayed and spayed females until 56 days of age, when there was a drop in responsiveness in the nonspayed rats, associated with rapid increase in uterine weight. This lack of increase in uterine responsiveness after gonadectomy, which is in contrast to the results in the other organs studied, does not mean that removal of the ovaries does not affect uterine weight. The difference between the mean weight of the normal uteri and those of spayed females at 14 days, 6 days after operation, was small, and more cases would be necessary to establish whether the difference was significant; but at all other ages the differences were apparent. The mean uterine weight in a series of 41 normal females at 26 days was 21.9 mg. and for 17 spayed females, 15.4 mg. At 36 days the mean weight for 43 normal females was 27.5 mg. and for 14 spayed females, 16.2 mg. At 56 days the mean weight for 55 normal females was 155.1 mg. and for 12 spayed females, 20.0 mg. From these data it can be seen that the uterus is normally a very slow-growing organ in the period of 14–36 days and that spaying results in a further slowing of growth.

It is possible that under such growth conditions and with a relatively small difference between the uterine weights in the normal and the spayed females an increase in responsiveness after spaying could not be expected. However, the difference in behavior may lie in some other factor, possibly associated with the action of the androgen on the uterus. As reported above, testosterone propionate treatment had no effect on uterine differentiation; and the increased weights seemed to be dependent upon simple growth in all parts of the organ, so far as could be seen. In all other organs testosterone propionate caused not only mitotic activity, as reflected in increase in cell numbers, but also increased cell size in the epithelium, functional differentiation, and production of secretion.

The uterus, as discussed above, was far less responsive to androgenic stimulation than the other organs examined. The difference between the female prostate and the uterus in this respect was striking. The minimum effective dose of 25 μg. for the uterus, however, was well above the threshold of responsiveness, and 10 μg. was below. An intermediate dose might have given detectable weight increases.

It might be well to distinguish between sensitivity of the organs to small doses of hormone and the ability to give

marked responses to large doses. The uterus provides an example of the difference; for, when nonspayed females were treated daily with 100 μg. of testosterone propionate, a maximum increase of 375 per cent in weight was obtained, and the maximum response in the normal male prostate with the same dose was only 114 per cent. This appears somewhat paradoxical. The point is that with graded doses in gonadectomized rats the male prostate was found to be responsive to 1 μg. daily and the uterus required 25 μg. (although an intermediate dose between 10 and 25 μg. might have been effective). When the male prostate was treated in the absence of stimulation from testis hormone, the maximum response was 917 per cent as compared with 335 per cent in the uterus in spayed females. A careful analysis is necessary to distinguish between sensitivity to small doses of hormone and reactivity to larger doses which are above the threshold of responsiveness. In addition, any study of the reactivity of organs to exogenous hormones must take into account all possible sources of endogenous hormone stimulation which might mask responsiveness.

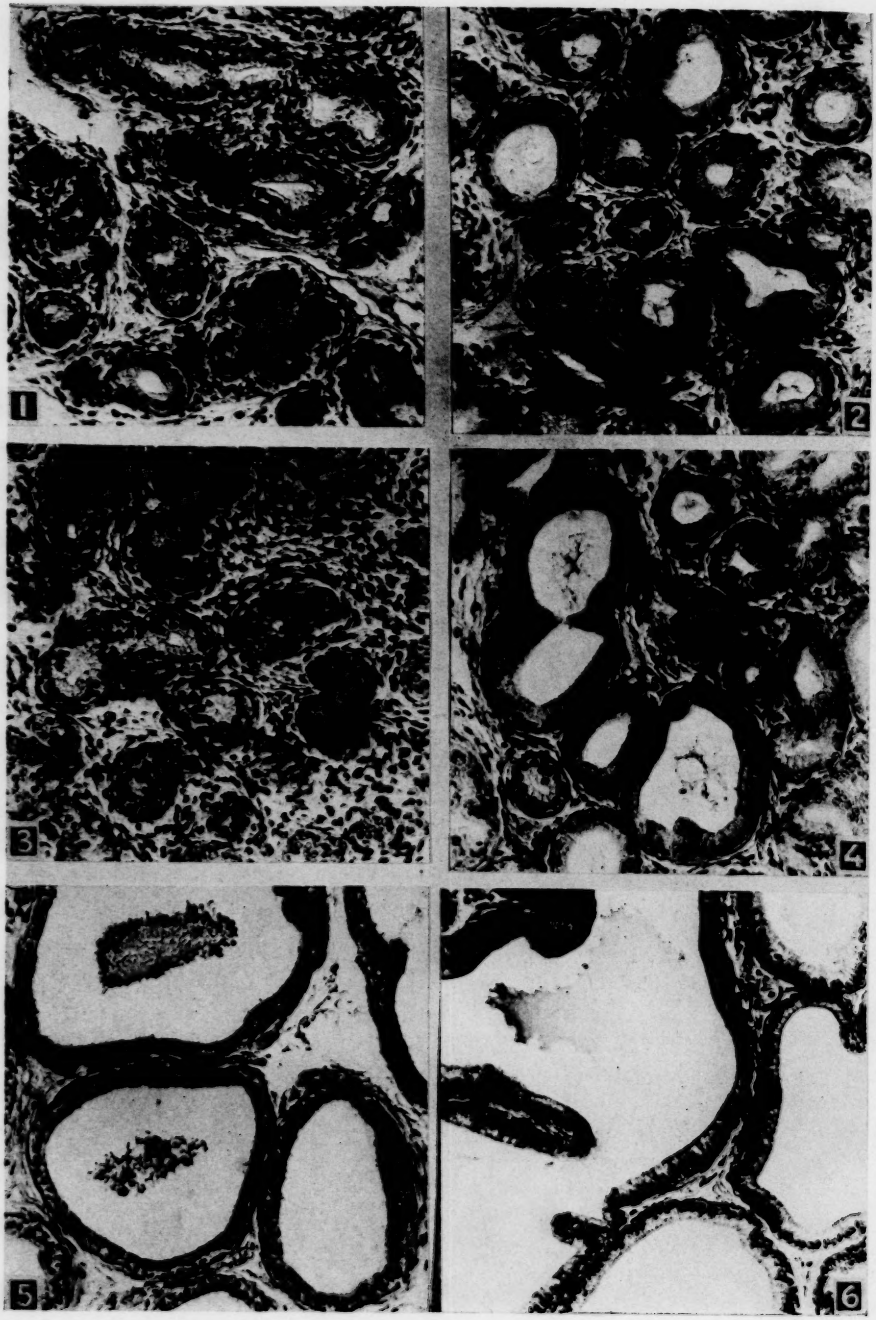
In a previous study (Price, 1944) it was reported that in nonspayed females the prostate was more responsive to treatment wth 100 μg. of testosterone propionate daily than the prostate of noncastrated treated males, as judged by the percentage-weight increase. This was found to be true in spayed females treated at certain ages with 100 μg. of the androgen daily, but with smaller doses no consistent difference was apparent. From histological criteria which determined the minimum effective dose in gonadectomized rats, the male and female prostate seemed about equally responsive.

From the data presented here and in earlier papers it is apparent that accessory reproductive glands and organs in male and female rats develop a high degree of responsiveness just prior to the time when puberal changes are about to occur. This reactivity was demonstrated by the ability of the organs to respond to relatively large doses of hormone by increasing in weight. The responsiveness attained at 26 or 36 days of age was maintained through 76 days in some organs and was somewhat diminished in others, possibly associated with the longer period of time between operation and treatment. The significance of this increase in responsiveness and the reasons for its consistent development in organs of both sexes is not yet evident. The relative importance of the amount of gonad-secreted hormone and the end-organ sensitivity in inducing the various changes associated with puberty needs further clarification. However, the changes associated with puberty and the age at which these changes occur depend upon many factors, some of which are genetic and some undoubtedly hormonal. The increased end-organ responsiveness in male and female rats just prior to puberty is an interesting observation which may prove to have fundamental importance when the basis for such a change in reactivity is found.

SUMMARY AND CONCLUSIONS

Male and female rats were gonadectomized at 8 days of age and treated with 1, 5, 10, 25, 50, and 100 μg. of testosterone propionate daily for 6-day periods beginning at 8, 20, 30, 50, and 70 days of age. The responsiveness of the male and female prostate glands, the seminal vesicles, and the uterus was determined by the study of weight changes and histological criteria. A marked increase in reactivity, as judged by percentage-weight change, was found in the

PLATE I



four organs at 26 days (uterus and seminal vesicle) or 36 days (male and female prostate). The responsiveness was maintained through the 76-day period in the prostate glands but was somewhat diminished in the seminal vesicles and uterus as age advanced. With the experimental procedure used, no increase in sensitivity to small doses was found with advancing age, nor did there seem to be an actual loss in sensitivity in the final age group at 76 days.

LITERATURE CITED

BURRILL, M. W., and GREENE, R. R. 1939*a*. Androgenic function of the adrenals in the immature castrate rat. Proc. Soc. Exper. Biol. and Med., **40**:327–30.

———. 1939*b*. Androgen production in normal intact and castrate immature female rats. *Ibid.*, **42**:764–66.

———. 1940. Further studies on the andromimetic function of the immature male rat adrenal. Endocrinology, **26**:645–50.

———. 1941. Androgen production in the female rat. The ovary and the adrenal in the immature rat. *Ibid.*, **28**:871–73.

HOOKER, C. W. 1942. Pubertal increase in responsiveness to androgen in the male rat. Endocrinology, **30**:77–84.

HOWARD, E. 1937. Is the X zone andromimetic? Amer. Jour. Physiol., **119**:339–40.

———. 1938. The representation of the adrenal X zone in rats in the light of observations on X zone variability in mice. Amer. Jour. Anat., **62**:351–73.

MIESCHER, K.; WETTSTEIN, A.; and TSCHOPP, E. 1936. The activation of the male sex hormones. II. Biochem. Jour., **30**:1977–90.

PRICE, D. 1936. Normal development of the prostate and seminal vesicles of the rat with a study of experimental post-natal modifications. Amer. Jour. Anat., **60**:79–127.

———. 1939. Normal development and regression of the prostate gland of the female rat. Proc. Soc. Exper. Biol. and Med., **41**:580–83.

———. 1944. Reactivity of the prostate gland of the female rat in relation to age. Amer. Jour. Anat., Vol. **75**, No. 2.

PRICE, D., and ORTIZ, E. 1944. The relation of age to reactivity in the reproductive system of the rat. Endocrinology, **34**:215–39.

SMITH, P. E. 1926. Hastening development of female genital system by daily homoplastic pituitary transplants. Proc. Soc. Exper. Biol. and Med., **24**:131–32.

———. 1927. Genital system responses to daily pituitary transplants. *Ibid.*, pp. 337–38.

SMITH, P. E., and ENGLE, E. T. 1927*a*. Experimental evidence regarding the role of the anterior pituitary in the development and regulation of the genital system. Amer. Jour. Anat., **40**:159–217.

———. 1927*b*. Induction of precocious sexual maturity in the mouse by daily pituitary homeo- and hetero-transplants. Proc. Soc. Exper. Biol. and Med., **24**:561–62.

PLATE I

FIG. 1.—Prostate of a 14-day-old male castrated at 8 days. Note the small acini. ×250.

FIG. 2.—Prostate of a 14-day-old male castrated at 8 days and injected 8–13 days with 1 μg. of testosterone propionate daily. Littermate to the control male in Fig. 1. Note the size of the acini and lumina, the high epithelium, and pale light areas. ×250.

FIG. 3.—Prostate of a 14-day-old female spayed at 8 days. Note the small acini. ×250.

FIG. 4.—Prostate of a 14-day-old female spayed at 8 days and injected 8–13 days with 1 μg. of testosterone propionate daily. Littermate to the control female in Fig. 3. Note the size of the acini and lumina, the high epithelium, and pale light areas. ×250.

FIG. 5.—Prostate of a 76-day-old male castrated at 70 days. Note the moderately distended acini but flat castrate type of epithelium. ×250.

FIG. 6.—Prostate of a 76-day-old male castrated at 70 days and injected 70–75 days with 1 μg. of testosterone propionate daily. Note the size of the acini, the high epithelium, and pale light areas. ×250.

SOCIAL BEHAVIOR OF THE AMERICAN CHAMELEON (ANOLIS CAROLINENSIS VOIGT)[1,2]

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WITHIN the last two decades, experimental studies have greatly broadened the foundations of a comparative social psychology of vertebrates, as part of the more comprehensive field of general sociology (Allee, 1931, 1938). At the same time zoölogists, particularly those concerned with the endocrines and with the nervous system, have shown increasing interest in the behavior of the organism as a whole.

Recent reviews which deal with special aspects of this subject (Noble, 1939; Crawford, 1939; Beach, 1942; Allee, 1942, 1943; Collias, 1944) have emphasized the phylogenetic significance of studies of lower vertebrates. The need for further description and experimentation has been especially evident in the case of the reptiles, which are considered to have branched from the same stem that gave rise to birds and mammals.

The present study grew out of the survey of lizard mating behavior by Noble and Bradley (1933). These authors found that space restriction markedly influences social situations; for instance, homosexuality, never observed in the field, was frequently recorded in crowded laboratory cages. In order to obtain a truer picture of the behavior of a typical saurian, we have used larger cages and have released the lizards within a spacious greenhouse. This procedure offered the advantages of controlled experimental tests while avoiding artificiality as much as possible. As a result we have been able to follow the rise and fall of breeding behavior and the claiming of territory under near-normal competitive conditions.

ANIMALS USED AND THEIR TREATMENT

"Chameleons" were purchased from Mr. R. H. Constant, of Thibodaux, Louisiana. Two hundred were usually ordered at a time; and each shipment represented a wide cross-section of the field population with regard to age, size, health, and sexual status. More than 1,500 lizards were brought into the laboratory; from these, specimens were selected for experimentation after observation in supply cages. Approximately 600 animals were used during the period of study—from February 2, 1936, to December 24, 1940.

[1] Aided by grants to the late Dr. G. K. Noble from the Committee for Research in Problems of Sex, National Research Council, and from the Josiah Macy, Jr., Foundation.

This investigation was performed under the supervision of Dr. Noble; it was terminated shortly after his death in December, 1940. The first-named author is solely responsible for the final interpretation of results and preparation of the manuscript. Two previous abstracts concerning portions of this material have been published (Noble and Greenberg, 1939; Greenberg and Noble, 1942).

[2] Mr. V. Sauvignet, Mr. A. Dury, and Mrs. M. Madof assisted at various times in making observations; it is a pleasure to acknowledge our indebtedness to them for much of the factual data herein presented. We also gratefully acknowledge the aid of Dr. W. C. Allee and Dr. Frank A. Beach in the organization and helpful criticism of the manuscript. We are indebted to Mr. Kenji Toda for drawing Figures 5–10, inclusive, from the original photographs.

Figure 1 is a scale plan of the experimental area, one room of a large greenhouse situated atop the African Wing of the American Museum of Natural History. A special helioglass roof permitted full utilization of sunlight, and a constant temperature of about 28° C. was maintained except during the summer months, when it frequently reached 35° C. As illustrated in Figure 1, the habitats ranged in size from small cages, such as were also used by Noble and Bradley (1933) and by Evans (1935-38), to the comparative freedom of the whole room. The dimensions of the room and the various cages are given in Table 1.

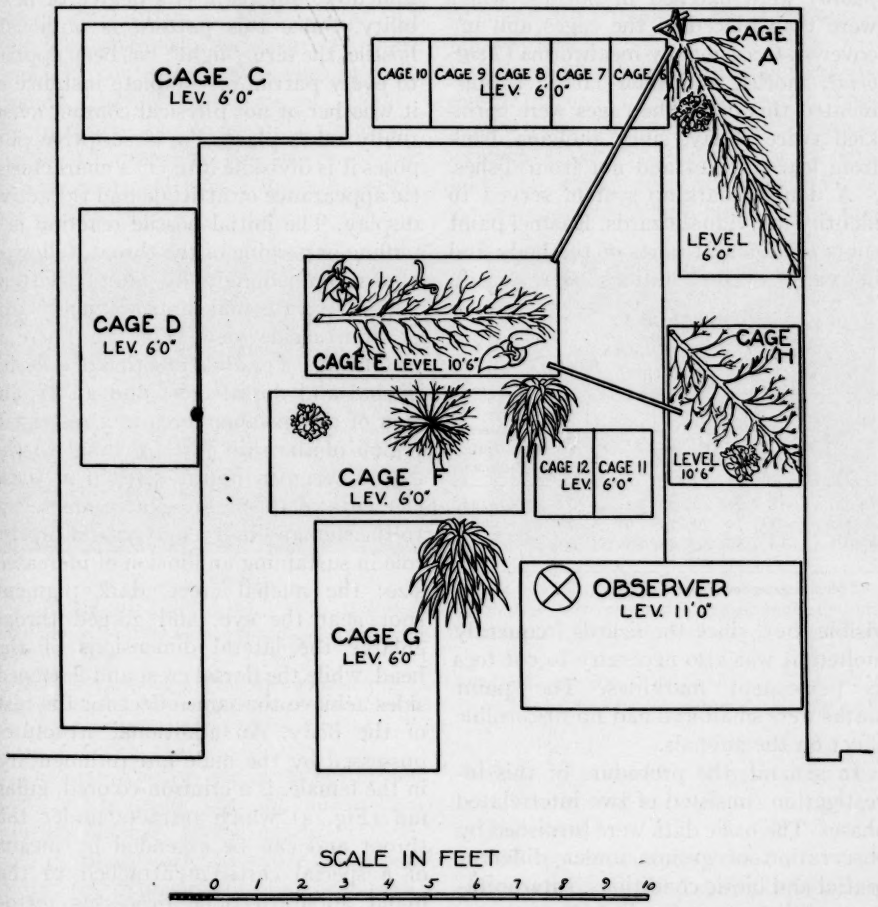


FIG. 1.—A scale plan of the greenhouse room in which the study was performed. Atop the cages are shown the branches, plants, and connecting sticks which served as habitats for the "free" lizards. The girders and metal window supports, which were also much used by the animals, are well illustrated in Fig. 16.

Each of the large cages contained two leafy plants and several crossed branches over which the lizards could climb. The attempt was thus made to simulate the normal field environment as far as prac-

ticable, although to some extent at the cost of convenience in observation. Feeding was accompanied by a minimum of disturbance; pupae of the blowfly (*Calliphora*) were hatched in bottles, which were then placed in the cages and uncovered. Occasionally mealworms (*Tenebrio*), moths, and their larvae supplemented this diet. The cages were sprinkled twice a day, since anolians drink from leaf surfaces and not from dishes.

A double marking system served to identify individual lizards. Enamel paint spots on different parts of the body and in various combinations were easily

TABLE 1

SIZE OF CAGES
(In Inches)

	Small Cages	A	C	D	E	F	G	H	Greenhouse Room
Height	18	42	42	42	32	46	42	96	86–222*
Length	24	70	70	70	69	70	70	43	206
Width	15	33	33	33	33	33	33	36	203

* Sloping greenhouse roof.

visible; but, since the lizards frequently molted, it was also necessary to cut toes as permanent markings. The paint marks were small and had no discernible effect on the animals.

In general, the procedure of this investigation consisted of two interrelated phases. The basic data were furnished by observation of groups under different spatial and biotic conditions. Interpretations of these records and indicated correlations were then tested experimentally whenever adequate tests could be devised.

THE BEHAVIOR PATTERNS

THE MALE

1. FIGHT PATTERN

During the breeding season (February through August), male anolians show hostility toward each other at almost every meeting. The pattern of display between males is sufficiently stereotyped so that it may be recognized without difficulty, but there is a degree of flexibility. Since this pattern is obviously hostile, the term "fight" has been applied to every partial or complete instance of it whether or not physical contact eventually takes place. For descriptive purposes it is divisible into (1) a characteristic appearance or attitude and (2) active display. The initial hostile reaction is a puffing or gorging of the throat, followed almost immediately by lateral flattening, so that the male appears much larger from a side view (Fig. 2). There is usually also a gradual erection of a fleshy nuchal and dorsal crest and an expansion of the melanophores in a restricted region of the skin just proximal to the eye. As seen in Figure 3, each of these elements of the fighting attitude seems to the human observer to have a precise role in sustaining an illusion of increased size: the nuchal crest, dark pigment spot near the eye, and gorged throat enlarge the lateral dimensions of the head, while the dorsal crest and flattened sides achieve the same effect for the rest of the body. An additional structure, possessed by the male but rudimentary in the female, is a crimson-colored, gular fan (Fig. 4) which retracts under the throat and can be extended by means of a special cartilage attached to the hyoid apparatus, the processus retrobasalis (von Geldern, 1919).

Many brief encounters were noted in which the dominant male did not erect the crest or show the black spot. These are under physiological control and have thresholds which are not too readily exceeded. The secretion of male hormone controls the development of the crest, as indicated by its out-of-season diminution and the ease with which it can be

stimulated by testosterone treatment (Noble and Greenberg, unpublished). At any season of the year mere handling can bring about the localized appearance of the black spot, as well as the general change from brown to green coloration. Kleinholz (1938) has demonstrated that adrenalin contracts, while intermedin expands, the melanophores of *Anolis*. The localized darkening of a patch of skin would appear to indicate a special nervous control of the melanophores of that region.

FIG. 2

FIG. 3

FIG. 2.—Initial fight reactions. The male on the left has flattened laterally and is displaying the dewlap, while the one on the right responds with the gorged throat and flattened sides but only the edge of his dewlap is visible.

FIG. 3.—A later stage of the same encounter. The left-hand male has assumed the full fighting appearance of gorged throat, flattened sides, nuchal and dorsal crests, and black spot on the skin in back of the eye. The right-hand male is not as fully aroused but is threatening with open jaws.

The male's active display is performed while maintaining the above-described fighting posture. It consists of (1) bobbing motions, with the entire body held stiffly; (2) extension of the throat fan; (3) parallel advance and retreat, circling the opponent and threatening him with open jaws; and, finally, (4) biting, sparring with jaws, and gripping the opposing lizard's snout or lower jaw tightly in an interlocking fashion.

The dewlap is never shown without accompanying bobbing movements. Analysis of motion-picture films reveals that this combined action has a distinctive rhythm. The fighting bob itself, when the dewlap is withheld, consists of five complete up-and-down swings; if the dewlap is also extended, it is pushed out after the second, held out stiffly, folded back and then followed by three additional swings.

Variability enters into the pattern in several places. The dewlap flourish may be omitted at any point when the action becomes close. The frequency of the fight bob varies with the reactions of the antagonist. When both males are thoroughly aroused, they repeat the same moves, and the fight may be greatly prolonged.

The sequence of a typical encounter between well-matched males can be outlined as follows: Upon seeing each other, both assume the puffed throat and flattened sides of the fight attitude and then advance, take parallel positions about 3 inches apart, and together move slowly up log or screen, alternately bobbing and arching their heads threateningly. First one, then the other, circles his opponent; and their heads, arching toward each other, come closer and closer

together. Retreat may follow advance, etc.; the climax is reached when both open their jaws widely and spar for a grip. Their heads are bent sideways, and they seize hold of the snout or lower jaw. The grip may be maintained for several minutes; during this time one or the other is punished and makes every effort to escape, often hanging completely suspended in the air. Sometimes it takes several such bites to reduce the spirit of one of the fighters. When he begins to back away, the aggressiveness of the other mounts perceptibly, and soon he is chasing the loser persistently. Later encounters are very one-sided; the subordinate is suppressed on sight and seldom again shows aggression toward this particular male.

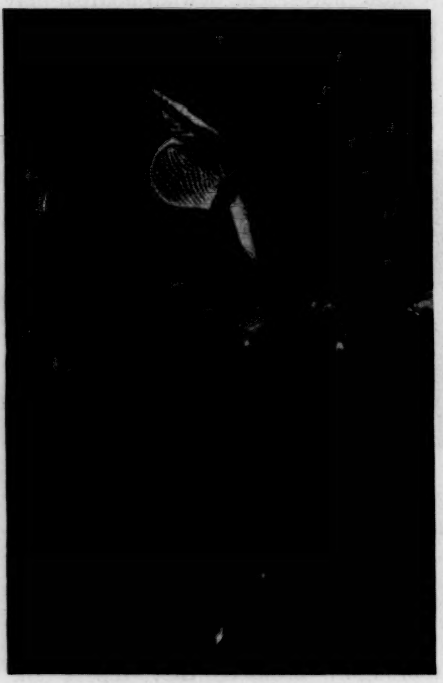


FIG. 4.—The display of the crimson-colored gular fan or dewlap.

A comparison with the fighting behavior of other iguanid genera as reported by Noble and Bradley (1933) may clarify the significance of elements of the *Anolis* fight pattern. The bluffing pose of *Leiocephalus schreibersii* closely resembles that of *Anolis* in regard to the swelling of the throat and flattening of the sides; the ridge along the back corresponds to the crest in *Anolis*. In discussing another genus of iguanids, *Chalarodon madagascariensis*, Petit (1928) states that the males display with "swollen throats" and erected dorsal crest very much as in *Leiocephalus*. This swelling or puffing out of the throat seems a common response in iguanids. The large dewlap of *Anolis* obscures the less conspicuous gorged throat even though the latter is a much more important feature of the fight pattern.

The morphological form of the crest varies. In *Basiliscus* it is no longer fleshy and erectile but spiny and fixed. In *Anolis* the crest is larger at the neck; and, together with the swollen throat and black spot in back of eye, the effect is almost to double the size of the head. This increase in lateral size is displayed by stiff bobbing motions, which we do not believe are aimed at securing a better view of the rival, as Noble and Bradley (1933) imply, but would rather seem to give the opponent a better view of the fighting pose. Dewlap display can be regarded as merely an added emphasis.

Behavior differences between genera are often accompanied by interesting structural modifications. For instance, *Sceloporus undulatus* does not patrol as large a territory as *Anolis;* and its fight pattern, while vigorous, is not as prolonged and active. *Sceloporus* shows its color on the sides of the body when these are fully flattened and the display is permanent, as compared with the intermittent flashes of color irregularly given by the more active *Anolis* males.

2. COURTSHIP AND MATING

A male beginning to court bobs several times and displays the dewlap; he approaches the female while bobbing with increasing rapidity, and this circumstance turns his advance into a prancing strut. Either the female is approached from the rear or she turns as soon as he moves toward her. She has ample time to run away, since there is both a preliminary challenge and at first a deliberate advance. If the female runs, the male may dash in pursuit; but often he does not. She is seldom caught if actively chased.

Throughout the performance there is no sign of fight—i.e., flattening of the sides, swollen throat, crest, or black spot in back of eye. The bob and approach differ from the corresponding parts of the fighting pattern, and the attitude is not hostile. Hence the courtship behavior and fight patterns are clearly discrete behavior systems.

It has been observed occasionally that the dominant male bobs and extends the dewlap in an attitude that is not courtship and yet does not seem strong enough to be considered fighting. Such reactions are perhaps intermediate but probably do not represent an intergradation between the patterns. They are more likely weak fight responses, since the throat is partially gorged and the sides are somewhat flattened.

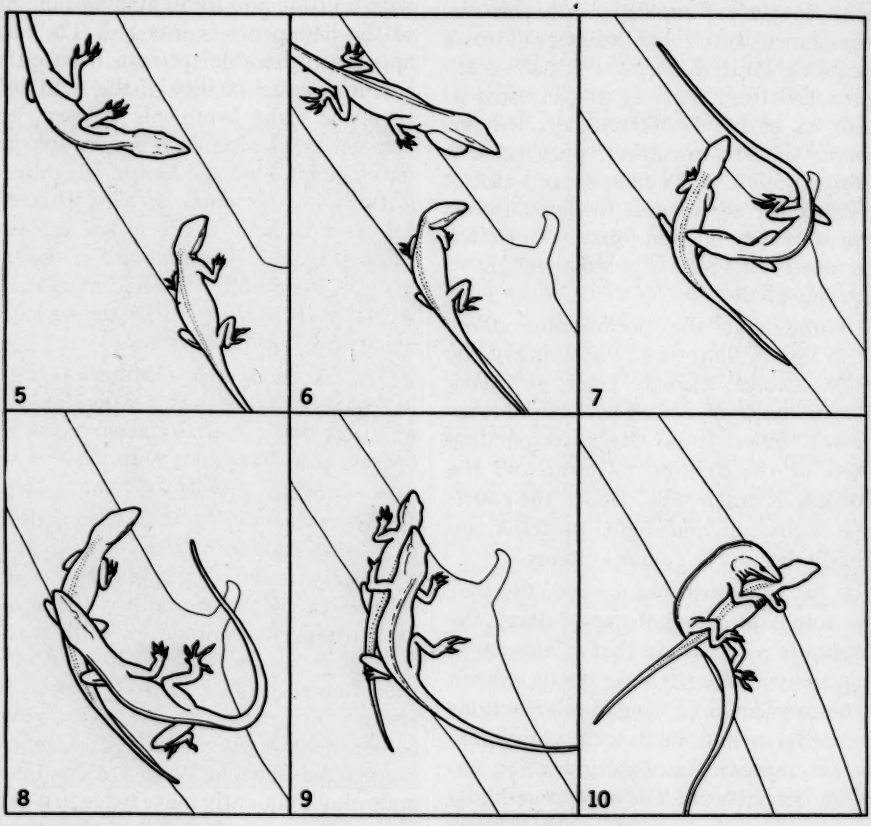
In the majority of successful matings the female stands in a receptive posture; the male mounts and grips the skin at the nape of her neck (Figs. 5–10). The remainder of the male copulatory behavior unfolds in a rather constant set of reflex reactions. He remains parallel with the female for a brief interval, during which she may nod several times. Abruptly, he doubles his body over one side, holding fast to the neck skin, and moves his tail underneath that of the female. Then he shifts his hind legs into a typical clasping grip on her tail-base; with a lifting and tightening motion, one of the hemipenes is inserted. The male appears to be able to mate from either side with equal facility; if the right side, then the right hemipenis is used, and vice versa. The leg clasp is accomplished with one hind leg, the foot of which rests dorsally on the female's thigh while her tail-base is held between his tail and thigh (Fig. 10). The pressure of the latter grip is probably the sole mechanism whereby the expansion of the vascular hemipenis is initiated.

The duration of the mating was timed in 81 cases. There was a range of 3–35 minutes; and copulation lasted, on the average, for 9.42 ± 0.67 minutes.

During the breeding season, and especially at its height, the diurnal activity of a male anolian on territory becomes a continuous round of fights and courtships. Some conception of the frequency of courtship and copulation is provided by Table 2. An impression is secured from these data of a regularity and persistence of mating drive which has never before been suggested for any reptile species. As shown in Table 2, a dominant male may frequently mate twice in a day, but rarely more often. Since the female lays a single egg at a time, the limits of increase of his offspring are set mainly by the number of receptive females within his range. Sexual selection thus has scope to effect the evolution of stronger, more aggressive males, equipped with more resplendent secondary sex characters. The striking sexual dimorphism in the American chameleon may have originated, in part, as the result of competition between the males for territory; but other factors, such as the safety of

the female in her small size and inconspicuousness (i.e., the regressive evolution of her secondary sex characters), can also be suggested.

but is overtaken, overpowered and ravished in the most brutal fashion." A reaction from this view is seen in Evans' (1935) conclusion that "the *mere flight*



FIGS. 5–10.—Line drawings of a courtship and mating sequence taken from a 16-mm. motion-picture film photographed by Dr. Frank A. Beach, of the American Museum. The male courts and approaches the female, who "stands" and arches her neck. Since they are facing each other on a narrow branch, the male passes her, circles to the female's rear, and mounts, while she co-operates with a slight turning of the body and stands. The completed copulation posture is shown in Fig. 10. Drawings by Mr. Kenji Toda, University of Chicago.

THE FEMALE

1. COURTSHIP AND MATING

The behavior of the female lizard in response to courtship has not been fully and accurately described in the literature. Wall (1922) believed that "she scuttles away as fast as she knows how, of the female from the performing male probably signifies acceptance of the male." Evans later (1938*b*) modified this statement by suggesting that the head nod is the main element in her "cooperation" in the mating.

The present study has furnished us

with a very different picture of the role of the female in courtship; this was primarily a result of the number of matings we have observed in detail. A total of 161 copulations involving untreated females were recorded during our study, and in 97 instances the initial reactions of both partners were witnessed. In 83 of the 97 cases the female stood still as the male approached her and passively permitted him to take a neck grip and copulate. A typical mating sequence is illustrated in Figures 5–10.

In addition, 32 instances have been observed in which the female showed receptivity and yet was not mated. The following case is quoted from our notes of April 30, 1937:

> Dominant male advanced toward Female 6 in courtship movement. Female 6 stood still and arched her neck. Instead of mounting, he courted around her. As the male circled, she moved to keep her back to him, head still bent. The male refused the neck grip and moved off, the female straightening as he departed.

Another example clarifies this point further:

> Female 2 remained in one spot and nodded whenever the male came near. He courted, she bent her head but he did nothing further. Later he courted Female 3 various times, then again approached Female 2. She bent her head even before he straddled. He mounted and gripped her, released the neck grip and secured a better one, then mated.

Evidently this male was not "ready" to mate until the second encounter with Female 2.

TABLE 2

FREQUENCY OF COURTSHIP AND COPULATION IN MALE ANOLIANS

	Single Dominance Cage C (Table 1)		Double Dominance Cage D (Table 1)		Free Anolians			Totals
			Series III		Series IV			
	Series I Dom. ♂	Series II Dom. ♂	North ♂	South ♂	♂ 7	♂ 1	Other ♂♂	
Observation periods	3/15/37 to 4/16/37	3/23/37 to 4/23/37	4/27/37 to 5/28/37	4/27/37 to 5/28/37	3/4–3/25/38 4/8–4/29/39	3/29–4/7/38 4/19–4/26/38	3/2/38 to 6/10/38	.....
Number of observation days.....	24	23	20	20	32	14	72	.....
Total number of recorded courtships	288	797*	329	243	148	65	390	2,260
Total number of recorded matings..	29	19	13	11	13	5	14	104

* Special effort made to record all courtships. Cage contained 1 male and 10 females.

COPULATION FREQUENCY IN A SINGLE MALE DOMINANT IN CAGE H (TABLE 1), 4/23/36 TO 6/2/36

	Date																		Total
	4/23	4/24	4/27	4/30	5/6	5/12	5/13	5/14	5/15	5/18	5/19	5/21	5/23	5/25	5/26	5/29	6/2		
Matings....	2	1	1	2	2	2	3†	1	2	2	1	1	1	1	2	2	1		27

† Only case of 3 matings in 1 day.

Although the typical copulation resulted when the male courted and the female did not move to escape, in 14 instances the females were observed to run and be caught. In 3 cases the female submitted after being pursued, but the 11 remaining cases were not so clear in this respect.

Thirty-two of the 161 copulations were seen among lizards permitted to roam freely about the greenhouse room. The behavior of the females just preceding these matings is listed in Table 3.

TABLE 3

COURTSHIP RESPONSES OF "FREE" FEMALE ANOLIANS PRECEDING COPULATION

Stood and arched neck	19
Stood	2
Ran; then stood and arched neck	2
Ran; then stood	1
Atypical	1
Not seen	7

The atypical mating took place when the female ran against the front of a cage and was trapped there in an attempt to penetrate the glass. The male gripped and took the mating position for 2 minutes; then she broke away. However, approximately an hour afterward the same male courted her again; and this time she stood and bent her neck. It follows that, although she ran the first time, she must even then have been approaching oestrus.[3]

The principal elements observable in the oestrous behavior of *Anolis* were the following: (1) standing to allow the neck grip, (2) the neck reflex, and (3) the head nod. The most critical response was that of inclining the head even before the male had touched the neck skin. It is doubtful whether this aided in guiding the male to a proper grip. Another clear sign was that the female "stood" for the male. This was not merely passiveness in the sense that she disregarded him, for there seemed to be complete "awareness," judging from the head nod and eye movements. When the female bowed her head even before the male came close, she appeared to be soliciting the mating. However, the male generally moved toward the female and did not court to draw her toward him.

The head nod will be discussed more fully in another place (p. 407). It is the usual response to the approach of a large male and has been observed even when the female ran immediately afterward. It is, therefore, not a critical sign, in that it does not identify the female as being in heat.

In order to test this concept of receptivity, females in breeding condition were spayed and their subsequent courtship and mating behavior was compared with that of unoperated controls; others were implanted with *Rana pipiens* anterior pituitary glands during the nonbreeding months and assayed with similarly implanted males. The technique was essentially that of Rugh (1937).

On January 7, 1937, 4 females and 1 male were implanted with 5 *R. pipiens* anterior pituitaries each; 1 week later the females received 3 more pituitaries and the male 5 more. These 4 females and an equal number of untreated controls were introduced into the male's cage. The first mating with an implanted female was observed 5 days later. From January 18 to February 9, 1937, a total of 9 copulations was observed, all with implanted females which showed the full seasonal oestrus. None of the controls mated at this time, although the breeding season was almost at hand.

[3] The terms "oestrus," "heat," and "sexual receptivity" are here used as synonyms; they refer to the behavior patterns which result from endocrine correlation between maturation of the ova and the condition of the central nervous system at the optimal time for mating. For further discussion, see p. 434.

Eighty-nine other females were similarly treated at various times, in connection with experiments to be described below. While test conditions such as the above were not generally maintained, the regularity with which these females came into full heat was very striking. A standard method of inducing receptivity consisted of an initial implantation of 3 *R. pipiens* pituitaries (anterior lobes), followed, after a week's interval, by 2 or 3 more. Several days after the second treatment, the females would usually show willingness to mate with treated males known to be sexually active.

The reciprocal experiment is to eliminate the ovaries either partially or completely and then compare the reactions of the spayed lizards with those of intact controls. Five healthy adult females were ovariectomized late in April, 1936. From May 5 to May 25, 1936, a total of 16 matings was recorded between pituitary-implanted males and 5 untreated controls, while the 5 castrate females in the same cage were not mated at all. On May 29, 5 new controls, operated upon in the same way but without removing ovarian tissue, were placed with these castrates. Four more matings took place, all with the controls.

Post-mortem examination of the 5 "spayed" females revealed that a portion of one ovary had remained and become somewhat hypertrophied in 4 cases. The amount persisting apparently had not been sufficient to maintain an oestrous level.

On July 21, 1936, 6 more females recently spayed and fully healed and 6 pseudo-operated controls were placed in a large cage with a pituitary-implanted male. One of the control females developed paralysis of the hind legs, and a normal uncut control was substituted. From July 21 to August 28, 1936, 8 copulations were recorded, 4 with this latter female and 4 with a single pseudo-operated control. This is understandable in view of the lateness of the season, when only scattered females come into oestrus. None of the castrates showed receptivity or mated. Autopsy revealed no macroscopic trace of ovarian tissue. Many spayed females were used in the course of other experiments; they were not seen to permit copulation at any time.

In 1936 the limits of normal mating were noted to be from April 15 to August 28. The following year a mating of an untreated female was seen on January 25 and another on February 15, 1937; thereafter copulations were frequent. The last was recorded on August 20, but daily observation had been stopped by the end of June. In 1938 the first mating was on March 15; breeding was almost entirely over by the beginning of August. These data are not sufficient to delimit the breeding season very precisely; but it is clear that, despite wide individual variations, the bulk of reproductive activity in our laboratory populations occurred during the months of February through August.

While the males were sexually active for long periods of time and copulated almost daily, the females varied greatly in this respect. Table 4 presents a comparison of the frequency of mating and oestrous display in 14 females which were observed from March 2 to June 10, 1938, in the comparative freedom of a large greenhouse room (see p. 414). Some mated often and showed consistent oestrus, while others were not seen to mate and were rarely receptive. Even the females with high mating scores were not predictable as to courtship responses; for example, Female 5 mated on April 13 but ran twice from the male during the

same day and did not mate again until April 27.

Besides the endocrine factors undoubtedly involved in this fluctuation, there were the less tangible effects of changes which tended to throw established relationships out of balance. The most common variations were: (1) displacement of males due to fights, accident, or sickness; (2) emergence of dominant females; and (3) environmental factors, such as light, weather, and food supply.

The effect of a complete change of males upon the behavior of the resident females of an established territory was tested experimentally with "free" anolians. In 19 trials it was observed that the females scatter and hide from a new male; after 2–6 days they gradually return to their accustomed places. During the period of readjustment the unfamiliar male induces flight reactions in most of the females, but the oestrous ones may "accept" him.

Two hundred and eighty-eight fights were observed among the 14 "free" *Anolis* females. Whenever one female dominated the others in the male's territory and consistently drove them out or forced them to hide, there was an obvious interference with their mating behavior.

The effects of light and weather were very noticeable. Bright sunlight brought a general stirring and bustling about, while rain and darkness had the reverse influence. No observations were made when food was scarce; there is a distinct possibility that even under mild starvation the reaction patterns of the lizards would have been different.

TABLE 4

VARIABILITY IN OESTROUS BEHAVIOR OF "FREE" FEMALE ANOLIANS
(March 2–June 10, 1938)

High Mating Scores (Group 1)			Intermediate Mating Scores (Group 2)			Low Mating Scores (Group 3)		
♀	No. of Matings	No. of Oestrous Signs*	♀	No. of Matings	No. of Oestrous Signs	♀	No. of Matings	No. of Oestrous Signs
5	5	10	9	2	1	46	0†	2
47	5	4	4	2	0	12	0	3
44	4	10	18	1	2	23	0	1
33	4	6	14	1	1	6	0	0
11	3	7	.....	.....	.....	16	0	0

* In addition to matings; i.e., standing or the neck bend.
† Neck-bite marks indicate one possible mating.

2. THE MALE'S "PREFERENCE" OR "CHOICE" IN MATING

It has been observed repeatedly that the males exercise some degree of "choice" in courting or mating one female rather than another. In one sense, every courtship represents a "choice" if other females are present. Relative availability as to distance and visibility are obvious elements which determine such preference. When one female is courted rather than another equally or more available, other factors must be in operation.

Examples of "preference" are numerous in our notes. A typical instance is the following:

March 25, 1938.—Male 7 courts Female 33. She nods and bends her head, but he continues

down the log and courts Female 23, 20 inches away. He then courts Female 18, who nods, stays, and bends head at instant he touches her neck.

For a possible explanation of the mating of Female 18 rather than Female 33, we need to consider the following records, taken the preceding day:

Male 7 courts Female 11, who stands and bends head, but he walks away. Male 7 courts various females; but when he straddles Female 33 and she flexes her neck, he goes on. Male 7 again courts Female 33, who stays and bends head, but he does not mate. Male 7 courts Female 11, she arches her neck, but he goes past her.

Evidently, at first the male was unable to copulate with the receptive females in his territory. The mating of Female 18 indicated that he had finally reached the point in degree of activation when he could complete a mating.

Still another factor is seen in the following instance, observed on March 23, 1938: "Female 42 was newly released. Male 7 sees her and rushes over, courting. She stands, bends head, nods; he straddles, moves off." The male responded differentially to the newcomer female immediately and was stimulated to court but was not sufficiently "ready" to mate. Later Male 7

circled back to Female 42, who had not moved. He courted, and she bent her head without nodding. He pranced around, almost took a grip, but did not. Afterward he found Female 6 close by and courted. She stood and bent neck; this time he secured the neck grip and mated.

This last courtship was less vigorous than his response to newcomer Female 42; yet he mated Female 6 apparently because he was aroused at the moment.

Recognition of sex of a newcomer female lends added drive to the courtship of the male at a time of rather low activation. The following case illustrates this point rather well:

March 31, 1938.—Female 44 (newly released) approached Male 1; she nodded and he strutted over and mounted. She stood, bent her head, and nodded, but he moved off; he came back and she stood again and bent head. He circled, approached, and mounted, while she stood perfectly still with bent head. Again he left her, although she followed a little ways. Male 1 returned to Female 44 shortly thereafter, and this time without more ado took the neck grip.

The male was attracted to the newcomer, was stimulated repeatedly, and finally mated.

A third factor in male "choice" is movement: "May 25, 1938.—Male 25 was resting a short distance from Female 47. He paid no attention to her but courted Female 31 when she came into his view at the limits of his territory." In another case: "Female 12 moved into view on a cross-branch. The male courted and strutted toward her; on the way he had to pass Female 47 but continued after Female 12." Motion attracts attention; and a moving female is courted, whereas a quiet female may be disregarded. Of course, if the male is sufficiently active, he will court any female and search out even motionless ones. "Choice" is important at low stimulation rather than at full rut. This appears to be a principle of rather general applicability; it is well illustrated in the male rat (Beach, 1942), in which the degree of sexual arousal is a product of two independent variables, excitability of the male and excitatory value of the stimulus object.

A fourth possible factor is memory of earlier unsuccessful courtships. If males are able to distinguish between individual females and remember previous courtships even for a short time, this might account for some cases of "preference." Repeated runs and chases after a particular female might excite the male sufficiently to mate. For instance: "April 21,

1938.—Male 7 courts Female 6, and she jumps away. Female 5 comes toward him and bobs, but he continues after Female 6." This supports the suggestion of Evans (1935) that the flight of the female is stimulating to the male; but probably it signifies relatively low stimulation at the moment on the part of the female.

3. EGG-LAYING PATTERN

Parental care in this species is limited to the digging of a nest and the concealment of the egg. The female burrows with her snout at about a 45° angle, and the forelegs sweep soil out as it is loosened. When the nest is in moss, the forelegs aid in parting the fibers. The principal role in digging is played by the poking movements of the snout. In several cases the whole process lasted approximately 3 minutes; the female then turned and placed her cloacal region over the hole. Extrusion of the egg and the accompanying stress and strain seldom took more than a minute, but in one case over 20 minutes were required to release an especially large egg.

The female regularly retains air in the lungs and becomes inflated; this perhaps supplies added pressure for oviposition. The egg either falls into the depression or is pushed in with the snout. Since the hole is dug at an angle, the egg may land right near the opening; it is pushed down and the female packs soil underneath, poking with her snout until the egg is raised on a firm foundation. Soil is then brushed into the nest with either foreleg alternately and tapped down with the snout. The egg is completely covered, and the hole is obliterated.

When the female dug in hard soil or in a clump of moss, the procedure was correspondingly modified. One female which laid on very dry and hard soil in a flower pot dispensed with digging entirely. She lay flat on the ground, extruded the egg, and pushed it into concealment between two lumps of soil. Then she covered it further with backward paddle movements of her forelegs. Several cases of egg-laying in peat moss (*Sphagnum*) have been observed. The female burrowed into the moss with her snout, going in deeply, the forelegs aiding in separating the fibers. The egg was pushed down; and, although efforts were made to close the hole, it was left partially open.

Among the "free" anolians the curious phenomenon was observed that several females dug nests at approximately the same spot. A number of eggs were taken from this one place. The seeming "communal" arrangement perhaps resulted because this spot was especially suitable for nesting, but the nature of this advantage was not noted.

One complete egg-laying of a Floridan *S. undulatus* subspecies was recorded in our notes on July 2, 1936. It is reported here by way of comparison. The female excavated a deep, crescent-shaped hole on a slant, burrowing completely underneath the surface. The forelegs were used to dig with, and the backlegs kicked the soil out. For a period of more than 15 minutes the female was entirely concealed.

Finally she stuck her head, all covered with soil, outside the hole and pulled herself out. She seized and ate a fly near the entrance and then re-entered, head first, and made sweeping movements with her forelegs and poking movements with her head as if packing the earth down. She turned around, entered tail first, and made backward paddle movements, first with right, then with left, forepaw. Then she repeated the movements with her head and shoulders in the hole, coming out again to make the backward paddle movements. When in the hole head first, she did not dig but made forward sweeps with the paws and packing movements with the snout. She stopped periodically for 10 or 15 seconds, as if to rest. After a while

she limited herself to backward paddle movements near the edge of the hole. . . . . Her interest continued for quite a while after all signs of the nest were eliminated, and she returned frequently to push soil toward it.

Four cases of egg-laying of *Basiliscus americanus* were observed during the course of the study. None of these were complete records because the laying invariably took more than 1 day. The female alternated periods of intensive digging (first the right foreleg, then the left) for 5–10 seconds with periods of rest and alert watching of the surroundings for a minute or two. The head went down, the female energetically dug with the paws, then raised up and rested. Stops of 10 minutes or more were frequent; several holes were abandoned and returned to. When the eggs had been laid, soil was brushed in and packed tightly by rapid, poking movements of the snout. The soil was shoveled in by forward pushing movements; the female then turned around or in a different direction than she had been facing and repeated the digging and poking. She turned in a circle, with short active periods and long rest periods. When the nest was filled, the turning smoothed and leveled it off, so that all signs were removed.

The strength and duration of the pattern in *Basiliscus* is illustrated by a case observed on September 24, 1936. A female basilisk laid 18 eggs in a large basket of soil but was interrupted before she could cover them, and the basket was removed to secure the eggs; it was replaced about an hour later.

The female returned and resumed her pattern where she had been interrupted. The nest had been disarranged and soil added, but she continued as if it had not been disturbed. She dug material from the surrounding sides and shoveled it onto the nest with her back legs, then packed it down and smoothed the entire area. The male jumped onto the basket and startled her, so that she moved away. The male bobbed several times in tremulous courting manner, but she did not get out of the basket. Five minutes later she had resumed her digging and polishing up, with the male still there. The male moved out of her way and did not continue the courtship.

Such persistence was never recorded for *Anolis*. It is notable that the extent and duration of the egg-laying pattern varies directly with the difference in ovulation in these genera; the female that lays a single egg has a much more limited pattern than the two others, which lay many eggs at a time.

4. AGGRESSIVENESS

Under the conditions of the present study we have found that female anolians fight rather frequently and at any time of the year, while the males fight mainly during the breeding season. In one large cage, 218 fight-contacts were recorded among 10 females within a period from April 31 to May 23, 1937. In a second cage, where the presence of 2 dominant males may have served to inhibit them, 9 females engaged in 146 combats from April 27 to May 28, 1937. Similarly, 14 "free" females participated in 288 observed fights from March 4 to May 15, 1938. These time intervals are all well within the period of seasonal reproduction. The above 642 records clearly disprove the suggestion of Evans (1938*b*) that females of *Anolis carolinensis* fight only when spayed or isolated.

The majority of fight encounters involved merely a posture, bob, and direct chase to drive the other away; but many more lengthy struggles between two fully aroused females also occurred. As in the male pattern, the throat is gorged and the sides are flattened. No crest is ever raised, since this erectile tissue appears to be absent in the female and cannot be stimulated with testosterone

treatment. The black spot on the skin proximal to the eye forms during the longer fights; and the miniature dewlap, almost rudimentary as compared with the male's, is frequently shown. Females perform the tense lateral advance, circling and maneuvering for a grip; but conclusions are reached more quickly than in the case of the males.

28 to May 3 but immediately thereafter she engaged in 19 fights until May 10.

Further comparisons are furnished by the records of the "free" females. In 21 instances involving 8 females, fights and oestrous display on the part of the same individual were recorded during the span of a 6-hour observation period. However, almost half of the total matings

TABLE 5*

RELATION OF FIGHTS TO MATINGS AMONG 10 FEMALE ANOLIANS
(During 6 Hours of Observation per Day)

Date	♀1	♀2	♀3	♀4	♀5	♀6	♀7	♀8	♀9	♀10
3/31	0	0	0	0	0	0	2	0	14	0
4/1	1	1	0	1	0	0	1	1†	8	0
4/2	0	0	1	0	0	0	6	0†	6	1
4/5	0	0	0	0	1	0	2	0	12	2
4/6	0	2	0	0	1	0	0	0†	3	0†
4/7	3	0	0	1	3	0	10	2	0†	1
4/8	1	0	0	0	0	0	5†	2	0	2
4/9	0	0†	1	0	0	0	2	1	0	0
4/12	0	0	0†	0	2	0	13	2	0	6
4/13	0	0	0	1	0	0	2	1	0†	1
4/14	1	0	0	0	1	0	0†	0	0	0
4/15	5	2	2	0	5	0	2	2	0	4
4/16	1	0	0	1	9	0	3	2†	2†	2
4/19	0†	1	0	0	5	0	1	4	0	0
4/20	1	2	2	0	2	0	4	0	0	1
4/21	0	0	0*	0†	0	0	0	0	0	1
4/22	0	0	0	0	2	0	1†	0	0†	5
4/23	1	0	0	0	7	0†	5	1†	0	0
Totals	14	8	6	4	38	0	59	18	45	26
Matings	1	1	1	1	0	1	3	5	4	1

* Cage 3×3.6×6 feet. † Mating.

Feminine aggressiveness affords little correlation with sexual receptivity or frequency of copulation. Table 5 indicates the distribution of the 218 combats mentioned above with relation to the time of occurrence of 18 matings. There is no evidence of either negative or positive correlations in these data. For instance, Female 7 fought 30 times from April 7 to April 12 and mated right in the middle of this short period. Female 9 fought 43 times until August 6, mated the next day, and did not fight again until 11 days later. In a second cage a peaceful female mated 5 times from April

occurred when the particular females involved were peaceful, and, in fact, remained so at least 3 days before and 3 days after these copulations. Using the above arbitrary time limits, an equal number of matings can be counted which were connected with varying degrees of overt expression of dominance behavior.

Thus, there does not appear to be either an inverse relation, such as Evans (1936*b*) suggested between the ovarian hormones and pugnacity in the females, or any direct correlation. Some decidedly receptive females never fought, while equally as many others did so. Fully

spayed females sometimes became very aggressive, while others, similarly operated upon, did not fight (Noble and Greenberg, 1941*b*).

Combativeness in the female is probably an expression of essentially the same territorial urge more strongly manifested by the males. Since it is so poorly correlated with sexual receptivity, it is possible that the hormonal bases for these phases of female behavior differ. Male hormone in the female would account for fighting behavior; but an extragonadal source seems required, inasmuch as spayed females also fight. It is more likely that aggressiveness, as a genetic trait, does not require hormonal activation; both sexes are innately aggressive, but the seasonal increase in testicular hormone in the male transforms mere antagonism into defense of territory.

SUBORDINATE GESTURE

Social integration among voiceless lizards takes place through the medium of gestures. Head nodding has been described in many iguanid genera; Barbour (1926) tells of large aggregations of anolians which he observed to be bobbing and spreading their dewlaps in the bright sunshine. These movements have never been studied analytically, in spite of their obvious phylogenetic import. They form a system of signs that apparently partially takes the place of the vocalization so prominent in birds and mammals.

There are four kinds of head movements in *Anolis:*

1. *Wavering.*—A very gentle vertical oscillation of the head as the lizard catches its insect prey. It is seen more frequently in the juveniles than in adults; the latter showed less hesitation in striking from a distance.

2. *The head nod* (subordinate gesture). —Consists of a series of four mild up-and-down motions of the head, displayed in response to the presence of a large or dominant lizard.

3. *The fight bob.*—A set of stiff and deliberate vertical motions of head, shoulders, and the rest of the tense body, performed by a fighting lizard, i.e., while maintaining a fighting posture (Fig. 11).

4. *The courtship bob.*—Begins with one or two sweeping nods with the head and shoulders and then typically becomes more rapid and vibratory. When combined with an approach toward the female, the result is a strutting, jerky advance (Fig. 12).

FIG. 11

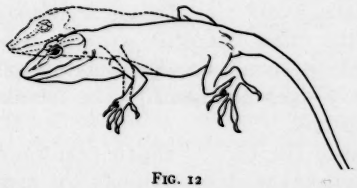


FIG. 12

FIGS. 11 AND 12.—Fig. 11 illustrates the fight bob, which is performed with the body held stiffly in the fighting attitude; the male rises up high on all four legs. Fig. 12 shows the courtship bob, in which there is a conspicuous absence of signs of fight and only the head and shoulders are raised by the forelegs.

Special interest centers around the head nod of *Anolis* because it has been interpreted very differently by recent authors. On the basis of physiological considerations Noble and Bradley (1933) assigned to it the function of an aid to vision. The nodding lizard supposedly receives multiple visual impressions on his retina, which work to reinforce the

image. Walls (1942) reiterates this same theory, for which no direct support is available. Evans (1938*b*) reached the conclusion that the head nod is the female's courtship reaction and is as far as she goes in "co-operating" in the mating. We find that, while the head nod is often, but not always, part of the female's response to courtship, it very definitely is not evidence of sexual receptivity. Not only females but also juveniles of both sexes and defeated adult males nod as a response to the presence or aggressive activity of a dominant male. Spayed females nod as readily as out-of-season females, but not as frequently as receptive ones; the latter are more often in the presence of the male and stand their ground while he courts them, whereas anoestrous and spayed females avoid him. Unless accompanied by the other definitive criteria already listed (p. 400), the head-nod response clearly does not identify the female as receptive.

Since the lizards which perform this gesture invariably submit to the aggression of the dominant male, one may interpret it as merely a sign of the performer's subordinate role in a dominance-subordination relationship.

ABERRATIONS

The spontaneous performance of the masculine type of courtship and copulatory pattern by the female has been recorded in some species of birds and mammals but very rarely in lower vertebrates. To our knowledge there is no report in the literature of its free occurrence in reptiles. However, treatment with crystalline testosterone propionate easily reverses the sexual behavior of female anolians (Noble and Greenberg, 1941*b*).

In the present study, out of some 700 brought into the laboratory, 20 untreated females were seen to attempt to copulate in male manner; in each case the full male mating pattern was observed up to intromission, which is obviously impossible. However, further study of individual cases led to the discovery of a second type of aberration, surprisingly enough present also in the males. Nineteen of the 20 females, 18 males of a total of over 800, and 4 other spayed and 4 pituitary-implanted females combined with their malelike courtships a form of vicious biting attack, which was so markedly different from the usual behavior of both sexes that it was at once termed "abnormal." In view of the novelty of this phenomenon in lower vertebrates, it seems advisable to present in slightly modified form excerpts from our records of two cases, a male and a female, which were observed for longer periods of time than any of the others.

August 12, 1936.—A female was observed to chase dominant Male 5 of Cage C. The female bobbed and then ran at him. The male first stood his ground and extended his dewlap; then, when she bit him, he turned and ran. The female attacked a second male, who flattened his sides and extended his dewlap several times; she walked away. This female was removed to Cage A for observation. (She will be called "Female A," and the erratic male will be called "Male A.")

Female A is chasing and biting both males and females. Her attacks are very sudden and usually result in the flight of her victims.

August 18, 1936.—Female A was transferred to a glass jar, together with a large male. A short while later the female had a neck grip on the male and was riding his back. Upon futher observation in the jar the female was seen to shift her hold and bob several times, but she did not proceed further with the copulatory pattern. She was returned to Cage A.

August 20, 1936.—A resident but subordinate male of Cage D, where two males held territory (see p. 413), was observed struggling with a castrated female with whom he succeeded in mating. A short while later he gripped another female, dashing at her without preliminaries. The female escaped.

Later this same male mated with a smaller male, put into the cage to test the ability of the alpha male to distinguish sex. He was observed struggling with this small male in the bottom of the cage, his jaws gripping him around the head. He then moved his tail into position and mated.

August 26, 1936.—Female A was observed astride and gripping another female. It was a typical neck bite, and she soon took the mating position. The pair struggled around the cage until the second female escaped.

August 27, 1936.—Female A was observed with a grip on the head of a large male. He finally succeeded in prying her off with his back legs and then bobbed and extended his dewlap. She was not intimidated and dashed at him. He dodged, then bobbed at her with open jaws. She moved off, bobbing in male manner.

August 31, 1936.—Male A gripped Female 2. There was no courtship, just a sudden rush. The female bit right back and twisted away, but the abnormal male rushed after her and seized her by the tail. She reached back and took a grip on his snout and they struggled around. The male then broke away; it was noted that this female resisted much more actively than a female usually does when avoiding copulation. Evidently she had been surprised and not courted.

Male A was seen to have a small male's snout tightly gripped in his jaws; he held on, bobbing. The small male made frantic efforts to escape.

Male A was seen with a large male's snout in his jaws. He held on for a long while, the large male unable to break away.

September 2, 1936.—Male A bobbed several times, then jumped at a small male and seized his left forearm. The male struggled and escaped.

Male A attacked another small male. The aberrant male is forced to stay at the bottom of the cage because the two dominant, active males drive him below whenever he ventures near them. The small males stay below for the same reason.

Male A was placed in Cage A in order to see how he would react in a different environment. He immediately attacked the residents in the cage, regardless of sex. He tried to seize a large male, which evaded him.

Male A was challenged by a male in fight attitude, who advanced upon him with sides flattened, etc. The normal lizard bobbed and extended his dewlap. The response of the abnormal one was as follows: He walked straight at the attacking male without showing any signs of fight whatsoever. Opening his jaws, he seized this male by the snout and held on. The pair struggled and fell to the ground. Ten minutes later the normal male managed to disengage himself.

September 4, 1936.—Female A was observed gripping another female on the neck and mounted on her back. She had been frequenting a clump of moss, where she rested when not active; and the captured female was maneuvered onto the moss, where the two stayed for several minutes. Then Female A carried her off and dragged her around; Female A stopped frequently and closed her eyes, breathing deeply. She shook the other female as a dog shakes a bone, then stopped, closed her eyes, and rested. Once she lost the grip and selected another hold, this time across the right eye, and hauled the female around that way into a patch of grass. By this time her victim was unable to resist further. After a minute's pause, she seized the female again; the latter was on her back, and the abnormal female seemed to be chewing on her head. She let go and licked her jaws; the hapless female was still on her back. After 2 minutes, Female A again took a grip, although on the ventral side. Her persistent activity was amazing. She released the dead female and moved off a bit, bobbing violently, rubbed her snout in the dirt several times, returned, and resumed her hold. A male came down the side of the cage toward her and she let go and rushed at him. The latter showed the fight reaction and opened his dewlap fully. She seemed intimidated and moved off.

The common elements in this aberrant behavior of both males and females were: (1) intense sexual excitability, even greater than that of dominant males (this activity was not affected by gross changes in environment or by handling: when placed in a different cage, the animal continued to attack or court; when put in a jar with another lizard, the reaction was given while in the jar); (2) no sex discrimination in the attacks or attempts at mating; (3) an atypical fight pattern (the fight attitude was occasionally assumed but was discarded immediately for a biting attack); (4) no

hesitation in attacking males larger than themselves and a greatly lessened sensitivity to the male's "bluff" or fighting pose; (5) a predominance of the courtship pattern or some element of it (the neck grip was the most common feature) (Fig. 13).

In general, the behavior gave the impression of an exaggerated courtship drive. A partial list of 28 holds taken on males or females shows that 17 were neck grips, 9 were on the snout, jaw, or

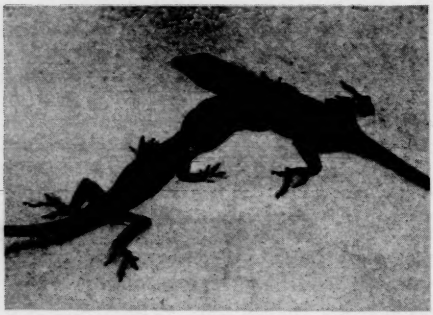


FIG. 13.—Juvenile male (abnormal) with neck grip on immature female. Note the head-epidermis lesions from defensive bites of other lizards and the evidence of failure to molt completely on hind legs and neck.

head, and 2 were on other parts of the body. The abnormal male mated completely while holding a grip on the head of a small male. Hence, most of the bites and attacks had some connection with the courtship pattern.

A total of 46 abnormal lizards was detected during 4 years of observation (1936–39). In the 6 months of most active mating (February through July) only 4 such males and 3 females were seen, whereas, in the other 6 nonbreeding months, 14 males and 17 females were found. Therefore, the peculiarity seems more closely connected with out-of-season physiology than with the breeding condition of endocrine or nervous systems.

In various experiments with spayed and anterior pituitary-implanted females, 4 of each kind became aberrant in behavior. Hence the ovarian hormones appear to be ruled out from direct causal relationship, while those of the anterior pituitary may possibly be involved in excess. Interesting accompanying symptoms were a blackening and formation of lesions of the epidermis of the head and failure to shed completely in that region. Although the lesions were in large part a result of the retaliatory bites of the victims, the skin seemed to be tender even in isolated animals. In general, the peculiar lizards shed more frequently and incompletely removed the old skin.

Since the thyroid gland is connected with molt as well as with activity, its hyperaction may be involved; but the behavior itself was predominantly sexual in type. Another alternative concerning which no evidence is available is that the abnormality originated in the central nervous system and was independent of endocrine influence.

DOMINANCE, SOCIAL ORGANIZATION AND TERRITORY

Morrow J. Allen's field notes on chameleons near Biloxi, ~~Missouri~~ Mississippi (reported by Noble and Bradley, 1933), and Evans' observations (1938*b*) on the related species *Anolis sagrei* at the Arnold Arboretum in Cuba indicate that male anolians space themselves widely on territories; as much as 300 feet may separate their lookout points. The importance of space is easily seen in the laboratory when a mixed group is placed in a small cage (Table 1). A single male becomes dominant and suppresses every other male resident. Under such conditions the dominant male has been stated to be unable to discriminate sex; for instance, Noble and Bradley (1933) observed a number of completed homo-

sexual matings, and Evans (1935) also found that in small cages a courting male pursues other individuals regardless of their sex.

Behavior in our larger cages furnished further evidence that males do not tolerate the close proximity of adult rivals within a limited area. An active adult male usually succeeded in dominating the entire cage, even though its volume was about fifteen times that of the smaller ones, and forced other males to hide under the shelter of plants or branches.

DOMINANCE AND WEIGHT

Whenever a group of males was introduced into a large cage, a short period of residence was invariably followed by a series of fights; at times as many as 4 males participated in such struggles. Table 6 presents the results with regard to the weights of the males in 24 staged encounters. In Trial A, 10 separate groups, comprising 51 males, were watched until a single individual had become dominant. Although each group was selected to give a wide initial weight range, only the final weights at the time of the emergence of a tyrant are given in the table. Equal feeding tended to decrease the ranges, and therefore the weights given are the more critical ones. The tests of Trial A were run at the very end of the breeding season. It may be noted that the 10 dominant males were not significantly heavier than the average of their fellows ($p = > 0.05$). Trial B, however, took place in March, when rivalries for territory are at their height. In 8 tests, totaling 49 males, the average weight of the dominants was significantly higher than the average of the rest of their groups ($p = 0.016$).

Another type of experiment is to remove the alpha males and permit successive dominants to arise (Evans, 1936*a*). We have performed this experiment with 15 males in a single large cage (Cage F, Table 1). All were re-weighed each time a new male became dominant, and the latter was discarded (Table 6, Trial C). Six males which emerged as tyrants under these conditions weighed significantly more than the average of the rest of their groups ($p = 0.03$).

In Trial B the dominant male was above the average weight of his subordinates 7 times and below it only once. On the assumption that this is due to chance, the alpha male in each trial would have a probability of $\frac{1}{2}$ of being thus heavier. From the binomial distribution we can calculate the probability that, out of 8 trials, the dominant will be heavier 7 or 8 times. The resultant p-value is 0.035. In Trial C the dominant male was always above the average of the rest of his group. The probability of this is $(\frac{1}{2})^6 = 0.015$. If we consider these two trials together, the dominant male was below the average weight of his fellows only once in 14 cases, an occurrence which would be expected on the basis of chance only 56 times in 10,000 ($p = 0.00056$).

These tests tend to show that during the breeding season the heavier males have an advantage over lighter rivals. Since weight is directly related to size, as well as nutrition, etc., and the larger males are also the older and perhaps sexually more mature ones, the underlying relation would seem to be that the more mature and experienced males tend to be the defenders of territory at that time. As Trial A shows, this does not hold true for the end of the breeding season. Perhaps the younger males retain their hormonal activation longer or come into rut later.

TERRITORY

During Trial A (1936) it was observed on several occasions that a secondarily

dominant male became active at the outskirts of the alpha male's holdings. A similar situation had been studied earlier that year in the tall Cage H (Table 1), in which two distinct and separate territories existed as a result of the complex arrangement of the plants and branches and the large size of the cage. Table 7 shows the intervals during which each male was tyrant and the number of times he was observed to copulate. Male 1, who held each of the territories at different times, was an atypical specimen. He had been treated with various hormones before he came into the experiment and, perhaps as a result, never copulated. His large size gave him a decided advantage until the smaller but sexually more vigorous Male 9 defeated him. The latter, in turn, gave way, for an unknown reason, to Male 13, who continued to dominate territory A until

TABLE 6

RELATION OF DOMINANCE TO WEIGHT* IN MALE ANOLIANS

TRIAL A. SEPARATE GROUPS OF MALES DURING THE NONBREEDING SEASON

	Date										Total Av.
	7/7	7/13	7/15	7/19	7/23	7/30	8/10	8/22	8/24	8/26	
Number in group	5	5	6	5	5	5	5	5	5	5	.....
Average weight (all but the dominant)	6.23	5.95	5.08	4.43	6.50	5.85	6.23	5.53	5.35	5.23	5.66
Weight of dominant	6.50	6.40	4.90	5.25	5.70	5.80	7.50	4.60	5.00	5.80	5.75
Difference	+0.27	+0.45	−0.18	+0.62	−0.80	−0.05	+1.22	−0.93	−0.35	+0.57	0.09

TRIAL B. SEPARATE GROUPS OF MALES DURING THE BREEDING SEASON

	Date								Total Av.
	3/8	3/10	3/12	3/15	3/22	3/22	3/29	4/1	
Number in group	5	6	6	6	6	6	6	8	.....
Average weight (all but the dominant)	4.95	4.28	5.28	4.80	4.82	4.30	4.90	5.33	4.82±0.155
Weight of dominant	6.40	6.60	4.90	5.00	6.50	4.80	6.40	6.00	5.83±0.278
Difference	+1.45	+2.32	−0.32	+1.20	+1.68	+0.50	+1.50	+0.67	1.01 (p=0.016)

TRIAL C. DOMINANCE IN A SINGLE GROUP OF MALES, FOLLOWING REMOVAL OF THE DOMINANT MALE OF PRECEDING TEST

	Date						Total Av.
	4/1	4/2	4/6	4/9	4/19	4/26	
Number in group	15	14	13	12	10	9	.....
Average weight (all but the dominant)	4.82	4.66	4.70	4.95	4.61	4.66	4.58±0.1824
Weight of dominant	5.60	5.70	4.90	6.20	5.80	5.30	5.58±0.225
Difference	+0.78	+1.04	+0.20	+1.25	+1.19	+0.64	1.00 (p=0.03)

* The weights used in this table were obtained not at the start but at the time dominance became firmly established.

September 15, 1936, when Male 2, the ruler of territory B, became dominant over the entire cage. The history of territory B was much simpler. Male 8 was aggressive and active there for almost 2 months but had to be removed because of disease. During the following 2 weeks

TABLE 7

DOMINANCE PERIODS OF 6 MALE ANOLIANS IN CAGE H, WHICH CONTAINED TWO DISCRETE TERRITORIES

Male	Territory A		Territory B	
	Periods when Dominant	Number of Matings	Periods when Dominant	Number of Matings
1.....	2/5/36 to 4/16/36	None	5/16/36 to 6/23/36	None
9.....	4/16/36 to 4/21/36	4	.....	.....
13.....	4/21/36 to 9/15/36	33	.....	.....
8*.....	.....	.....	3/19/36 to 5/10/36	9
10.....	.....	.....	5/11/36 to 5/16/36	5
2.....	.....	.....	6/23/36 to 9/15/36	6

* Removed because of cloacal infection.

two males (Nos. 10, 1) attempted to hold this territory but were finally driven out by large Male 2; the latter divided the cage amicably with Male 13 for almost 3 months.

In 1937 this kind of "double dominance" was studied in a different type of cage; the greatest dimension of this cage was its length, and the interior composition consisted of two symmetrical arrangements of plants and crossed branches distributed to favor the establishment of two territories. The first observed case was detected in Cage D (Table 1), as an outgrowth of one of the weight experiments. The preliminary steps in its development were not seen. The two males showed a very clear respect for boundaries; they often threatened each other but never actually came to grips. When chasing a subordinate male or courting a female, the limit of the chase was the boundary line, which seemed to be recognized with some exactness. The two fought with other resident males, courted, and displayed the dewlap within sight of each other. Hunger sometimes led to a brief intrusion by one male, who crossed over to snatch flies out of a jar in the other's territory; but after feeding he always retreated. In other cases where the line was crossed, the mere approach of the resident male caused withdrawal. An example of such an encounter is the following:

> The north male, in full fight posture, sidled onto the log where suppressed Male 2 was resting, near the border but somewhat more in the south male's half. This time he did not respect boundary, but went about 6 inches into the south half, always keeping an eye on Male 2. In the meantime the south male had driven suppressed Male 3 away from another part of the cage. He advanced in full fighting attitude down the center log toward the north male but did not actually attack. The latter started retreating very slowly and so did the south male; i.e., they were both going away from each other at a snail's pace, looking backward over their shoulders. When they were about 2 feet apart, each went on individual business, relaxing the fight attitude.

Here both males had just driven off large suppressed males but retreated from each other while still in fighting pose. In fact, the south male retreated while still in his own territory.

Further data on the courtship activity of these two males are given in Table 2. They were fairly equally matched with regard to sexual excitability and fre-

quency of copulation. Two other similar cases occurred spontaneously, although we experienced some difficulty in duplicating them experimentally. When a group of males is introduced into a cage arranged to encourage double dominance, one male often is sufficiently aggressive to rule the entire cage. Just how division into territories comes about was not observed; but it may be supposed that, after a long fight, mutual respect induces each to stay in half the available space.

Two additional cases were brought about by dividing Cages D and G with glass or cardboard until a male became dominant in each half, whereupon the partition was removed. Double dominance resulted only after repeated trials, since one male usually suppressed the other. The partition served merely as an experimental aid.

In the following breeding season (1938) it was decided to give still more room to a mixed group of lizards by letting them run free in the greenhouse; we had often noticed that escaped lizards managed very well and were healthy specimens. They made their home atop the cages and on the window rafters and fed upon flies or moths which were accidentally released. To accommodate the newly freed animals, some plants and branches were arranged, together with bridging vines and sticks (Fig. 1). These were sprayed with water several times a day, and flies were provided as food.

On March 2, 1938, the lizards which were already free within the experimental area were caught and marked; 5 such males and 5 females were again released where found. New lizards were added at intervals until March 31, when a total of 12 males and 24 females had been introduced. These animals were observed without limitation on their free distribution other than the arrangement of the general area as to food, water, light, and physical composition (branches, plants, etc.). The latter kinds of restrictions are, no doubt, also found in nature; the environment offered did not depart too greatly from field conditions.

By March 10 the situation as to territories of the males and the distribution of the females had stabilized sufficiently to permit certain comparisons. Male 7, a newcomer, defeated one of the old residents (Male 1) and took full possession of a large area; he patrolled the tops of Cages E and F and made occasional visits to Cage G. After a great many fights, Male 3, an old-timer, succeeded in maintaining a territory over Cage H until March 22 but then was beaten by Male 5, who had been his subordinate in all previous encounters. Still a third, Male 6, new to the experimental area, took up residence over Cage A, although no branches or plants were placed there until March 24. These males and several others, which will be mentioned below, were under fairly constant daily observation from March 2 to April 29, and their largely unrestricted behavior afforded us with an unusual opportunity of studying the establishment of territory and its defense against both newcomers and neighboring males.

In general, the same kinds of relationships already noted in the double-dominance cages prevailed when the lizards were freed in the greenhouse. The complexity of the physical conditions and the greater liberty to roam about gave much more scope to the interactions of males and females. For instance, the position of the subordinate male was completely transformed from that in a cage. A beaten male did not necessarily lose his sexual activity or all of his territory. Male 7, defeated by Male 1 on April 1, nevertheless succeeded in retaining part of his area and became

dominant there. Also, whenever a male was removed experimentally from his place, another was available on the outskirts to replace him.

TERRITORIAL HIERARCHY

As a result of this behavior of the beaten and interterritory males, there was noted on three separate occasions a hierarchy of at least three ranks, entirely dependent on modified territorial relations. When one male extended his control to part or all of a neighbor's area by defeating him (2 cases), the loser remained dominant, nevertheless, in his home area but cowered or hid whenever the other approached. Again, a newcomer or intruding male sometimes became active in part of a complicated territory and assumed the full appearance of dominance toward other males but ran from the slightest threat of the real master of the region.

Figure 14 illustrates the situation in the first observed case. On March 8 a very active fight occurred between Male 7 and Male 3 on the latter's territory. Male 3 fell to the floor during this encounter, and Male 7 withdrew to Cage E. Shortly thereafter Male 3 returned to his place and challenged formerly beaten Male 5 as if still dominant there.

Later the same day, Males 7 and 3 challenged each other from a distance of about 12 feet. Male 7 approached to within 5 feet, well in Male 3's domain. Male 3 did not attempt to drive him off. A few days earlier, Male 7 had severely trounced Male 1 on the latter's home ground. Shortly after the above encounter between Males 7 and 3, Male 1 entered Male 3's territory. Male 7 first threatened Male 1 from Cage E, then crossed the bridge and drove him out of the area; all this took place while Male 3 remained quietly on his vantage point.

The following day, Male 7 was completely at home on Cage H and Male 3 was out of the territory, only to return as soon as 7 left. Male 3 threatened Male 5 and chased him out. On March 10 a new male (Male 10) was introduced. The following record shows the kind of situation that existed the next day:

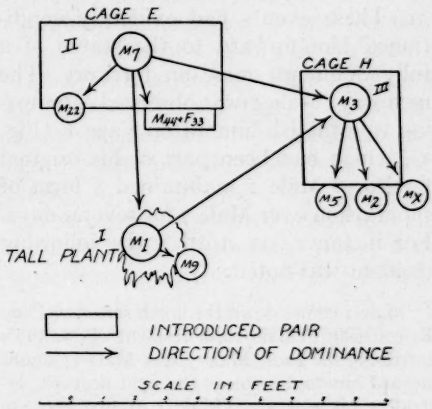


FIG. 14.—Relation of dominance to territory in male anolians. Male 7 was able to dominate Male 3 and Male 1 even on their own territory, which the latter still defended against other males.

Males 3 and 10 and Females 33, 11, and 12 are all on Cage H. After 20 minutes, Males 3 and 10 fight. They come to grips, and there is a sharp click of the teeth; Male 10 breaks away and runs. Thirty minutes later, Male 7 jumps to Cage H and slowly ascends to its vantage point. He comes across Male 10 on the lower part, challenges him, and Male 10 runs; Male 7 comes up a little higher, very aggressively. Male 3, instead of defending, jumps out of danger. As soon as Male 7 goes back to Cage E, Male 3 comes down and back onto a branch. He challenges young Male 2 and chases him down the cage.

However, 2 days later, Male 7 had abandoned Cage H to Male 3: "Male 7 makes no attempt to patrol *all* of the area he had Friday. He has seen Male 3 court Females 4 and 12 and does nothing about it." This tolerance persisted thereafter.

A second instance of "territorial hierarchy" originated as the result of experimental interference with Male 7's territory. On April 1 he was caged, whereupon Male 1 took his place. After 2 days, Male 7 was released but was decisively defeated by the now resident Male 1. Male 1 had defeated Males 10 and 5 and also mated with Female 44 on March 31. These events had evidently conditioned him upward to the status of a fully dominant male on territory. The next day, Male 7 was observed attempting to establish himself on Cage F (Fig. 1), which had been part of his original territory. Male 1 maintained a form of supervision over Male 7 for several days. For instance, on April 5 the following incident was noted:

> Male 1 comes down the north screen of Cage E, courting Female 16, a resident of Male 7's territory; she runs. Male 7 sees Male 1, comes toward him and assumes the fight posture, defending his new area. Male 1 at first retreats slightly, then holds his ground. They are 20 inches apart and bluffing. Male 7 retreats.

Action quieted down between the two for several days and then resolved into the usual relation between neighboring males who mutually recognize each other's rights and boundaries. This case was not as complete as the preceding one, since no third male was seen to threaten Male 7's hold on his new territory. It resulted in a two-rank system only but shows how the chain of interactions can start toward a three-rank system.

Another case which actually resolved into a three-rank hierarchy was observed on March 15. The notes of that day yield the following statement:

> Male 7 comes down to Cage F and is able to make Male 22 (newcomer) quiet down by threats; there is no real fight. Male 22 has been here for 2 days and has been gradually assuming dominance. He has beaten Male X and Male 5.

The fourth instance was first noted on March 14, in Male 6's territory on Cage A. Male 32 and an accompanying female were newly released there. Until March 23 the two males seemed to divide the outside of the cage peacefully, Male 6 on the south half and Male 32 on the north half. On that day the observer placed Male 32 in Male 6's half. The latter gave no reaction. A short while later, a new male was released near Male 32, who immediately showed fight. Then Male 6 came to the top of the cage and showed active fight, threatening the new male with much dewlap display. Male 32 quieted down, as if subservient to Male 6. The latter chased Male 32 out of the branches on Cage A. He also threatened the new male again, and the latter cowered. On March 25 it was again seen that Male 32 remained aggressive on the north half of the cage but ran from Male 6. The latter spent a great deal of his time on the other side.

These cases arose through competition among the males for territory. Three of the four represent a temporary hierarchical organization on a higher plane than the two-rank system involved in simple dominance. It is possible that more permanent three-rank hierarchies may exist among lizards in the field, but under our conditions they eventually resolved into either mutual respect of boundaries or disappearance of the second-ranking male.

FEMALES AND THE SOCIAL GROUP

The distribution of the females with relation to the territories of the males was studied both in cages and free in the greenhouse. A group of 10 females was observed from April 27 to May 28, 1937, in Cage C (Table 1), where 2 males held separate territories. Three young males were also present and were dominated by the 2 older males. The females ar-

ranged themselves very unequally; 2 took up residence in the south end of the cage and were joined by several occasional wanderers. The north side normally contained a minimum of 6 females. Other sets later introduced into the same cage were distributed much more evenly. Of the 2 females resident in the south territory, one fought more than any other female in the cage, the other the least number of times. The former's fights were spread fairly uniformly through the period of observation, and she was mated 3 times. The second female fought only twice and was mated the same number of times. In the adjacent territory the females alternated in dominance through the period: (1) Female 10 won 12 encounters with various others on two successive days, April 29 and 30, and ceased to dominate thereafter; (2) Female 7 began on April 30 and until May 10 had won 23 fights, after which time her victories were few; (3) from May 17 to 27, Female 6 fought and won 23 times, although she had not been active at all before that. Thus, 3 females alternated in fighting successfully and dominating in that territory. The other half of the cage showed dominance even more clearly; Female 2 fought off the wanderers and ruled over Female 3 and the young males which usually stayed in her vicinity.

An alternate dominance was observed also in another large cage patrolled by 1 very aggressive male. Table 5 shows that (1) Female 9 won 43 fights from March 31 to April 6, then won only twice to the end of the period; (2) Female 7 fought successfully 32 times from April 7 to 13; and (3) Female 5 began on April 15 and to April 23 had shown dominance 30 times. The size of the cage and the fact that it was one territory allowed for many other combats during this period, but the concentration of victories by these females in roughly equal periods during the month of observation points to territorial dominance. This view is strengthened by the fact that the females were never defeated during their dominance periods and showed a marked tendency to fight in and defend their usual locations in the cage.

During seasonal reproduction, the social group (1 male and several females) seems to be kept together by the male's activity in a territory. As a consequence of this bunching-together, contacts between females are more frequent and fights more numerous than at other times.

The females wandered much less extensively than the males. When 2 or more females restrict their activities to 1 male's territory, fights among them may represent a tendency toward monogamy. A male marks off a territory and evicts all his rivals, while 1 female in his territory tends to drive out her rivals and to keep newcomer females away. However, dominance is by no means as strongly expressed in females as in males. A dominant female is more likely than the male to tolerate others and does not usually succeed in keeping them out for very long.

Among the groups of "free" *Anolis* females, dominance was very clearly evident. They distributed themselves among the various male territories and became resident at a particular spot. In most cases they wandered less than the males. Table 8 shows the relationship between the number of fights won and their "residence periods," which are quite arbitrarily defined as any stay in a particular male's territory which lasted 4 days or more. The length and number of such periods varied, but it may be seen from the table that 277 of the 288 total fights were won by such resident females. This strengthens the hypothesis

that the fights took place in relation to a particular area; i.e., they were disputes over territory.

"Sexual territory" may be defined as "residence in a restricted area and its defense against others of the same sex." Where such defense by a female was so vigorous that others were not permitted at all, the female was considered as showing a territorial drive similar to that of the sexually active male. Female 14 so defended Cage H from March 24 to April 12, 1938. Dominance by the female was usually, however, not as complete as in the male. The breeding group consisted of as many as 7 females or more within the vicinity of a single male. There was most often 1 dominant female but there were occasionally 2. Others of the group fought among themselves or against newcomers, so that a "pecking order" among the females of one male's territory was not infrequent. Figure 15 illustrates 1 such relationship seen on April 9 among the "free" females. Although Female 5 was mated by the male, she was at the bottom of a three-rank hierarchy, as indicated in the diagram.

Another clear case was witnessed on May 21, 1938, also in the freedom of the greenhouse room. The following fights occurred in succession: "Female 12 defeats Female 46, then Female 4 defeats Female 12 and Female 47. The latter

TABLE 8

RELATION OF FEMALE RESIDENCE TO FIGHTING IN *Anolis*

(Number of Fights during Residence Periods of 4 Days or More)

♀	Total No. of Fights	First		Second		Third		Fourth	
		Residence Period	Fights	Residence Period	Fights	Residence Period	Fights	Residence Period	Fights
46	33	3/31–6/10	33	.....	.....	.....	.....	.....	.....
44	18	3/31–6/10	18	.....	.....	.....	.....	.....	.....
5	65	3/2 –3/22	16	3/28–5/3	49	.....	.....	.....	.....
23	41	3/15–3/18	.....	3/23–6/10	41	.....	.....	.....	.....
33	2	3/11–3/21	1	3/22–4/7	.....	4/13–6/8	1	.....	.....
47	8	4/1 –4/11	.....	4/21–6/10	8	.....	.....	.....	.....
14	30	3/24–5/13	30	.....	.....	.....	.....	.....	.....
11	37	3/8 –3/29	6	4/7 –5/13	15	5/16–5/31	9	6/7 –6/10	4
12	2	3/11–3/19	.....	3/21–4/7	1	4/11–5/5	.....	.....	.....
9	11	3/4 –3/10	.....	3/24–4/29	10	.....	.....	.....	.....
4	24	3/14–3/21	12	4/11–4/14	.....	5/6 –5/17	7	.....	.....
18	14	3/24–3/29	4	4/5 –4/14	10	5/9 –5/12	.....	5/31–6/6	.....
16	2	3/28–4/13	1	4/19–4/25	.....	4/29–5/4	.....	.....	.....
6	1	3/24–3/31	.....	4/21–5/2	1	5/12–5/18	.....	.....	.....
Total (14)	288*	.....	121	.....	135	.....	17	.....	4

* Total: 277 fights while resident in an area and 11 fights outside this territory.

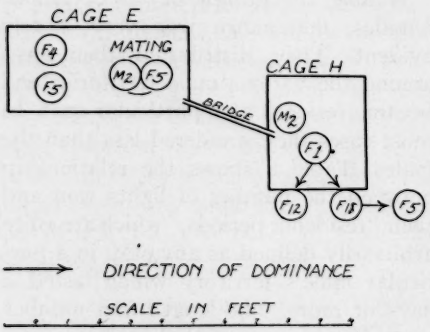


FIG. 15.—Relation of dominance to territory in female anolians. Within the territory of Male 2, temporary straight-line hierarchies were frequently observed among the resident females.

wins over Female 12." Since males were absent from the area at the time, these fights appeared not to be correlated with breeding unless it be assumed that the area itself was still connected with satisfaction of mating drive.

Consistently resident females did not necessarily fight strongly in all cases. The presence of an especially aggressive female in the same area was one inhibiting factor. However, the appearance of a newcomer frequently initiated a revolt by one of the quiet females, which sometimes established a new tyrant.

TERRITORY VERSUS THE ISOLATION REACTION

As the breeding season waned, a gradual diminution in the sexual excitability of the males was noted. They became lethargic and no longer patrolled the full extent of their territories. The time when this began varied, but the slackening was quite general by August and September. The females also became more scattered in their distribution and tended to avoid contact with each other and with the males. Antagonisms nevertheless persisted, in cages as well as in the greenhouse room. Fights between females were seen more frequently at this time than between males. The latter seldom met, but the females appeared to wander across each other's paths relatively more often.

In the period of January 19–31, 1939, we studied the behavior of lizards that had remained at liberty all winter in the greenhouse room. It was found that each of the principal male territories previously studied was occupied by a dominant male, who courted the resident females. The situation differed from that seen the previous March only in degree. The males were not very active; but, when tested by shifting each male into another's area and also by introducing newcomers, typical territorial defense was evoked, which was successful in each instance.

Isolated males were also observed on ledges and rafters dispersed throughout the greenhouse. These reacted to introduced test males only when directly approached within close range. The isolated males were not defending a territory but only their isolation area, a very limited region around their usual sunning spot. Occasionally a male and a female were observed together on the same ledge. There was no courtship but only a mutual toleration. These relationships between isolation and territory are summarized in Table 9.

In addition to strictly isolated male and female individuals and the pseudopairs mentioned above, the three main territories at this time each contained a number of females. However, no 2 females were together on a branch; all tended to be widely spaced and to avoid contacts. In such groupings, one female was seen to fight more actively than the others, not only on her vantage point but also within a radius of several feet. Female 2 fought on the greater part of Cage E, driving out Females 13, 14, and 15 at various times. Similarly, Female 6 fought on Cage A within a large radius of her lookout point. This behavior appears to differ from that of the breeding season only in lessened intensity.

Responses of a nature similar to nonbreeding isolation can be induced very readily by isolating individuals in small cages. The results thus secured need to be evaluated according to the calendar time when the study is made. For instance, the case history of small Male 8 may be cited (Table 10). Tests of the responses of this isolated male to lizards introduced into his cage were begun on November 29, 1937, and continued through February 21, 1938. Antagonistic

TABLE 9

CORRELATIONS BETWEEN THE TWO TYPES OF TERRITORIALITY OBSERVED IN *Anolis*

	Isolation Area	Sex Territory
Breeding condition.	Low androgen or estrogen level	High androgen or estrogen level
Comparisons in the male.....	1. Small retreat defended against males at short range 2. Contains no beta males 3. Females tolerated, leading to pseudo-pairing 4. Scatters individuals and satisfies asocial tendency	1. Large conspicuous area defended by constant patrol 2. May contain beta males but usually spaces males on area 3. Frequent courtship display at vantage points may bring females toward the male 4. Aids identification of sex and gravid females 5. Prevents weaker males from breeding
Comparisons in the female.....	1. Small retreat defended against females and juveniles but subordination to larger male may effect a pseudo-pairing 2. Contains one dominant female 3. Scatters individuals and satisfies asocial tendency	1. A part of male's territory defended against females, but courtship activity of male may hold several females together 2. May contain a straight-line hierarchy of females 3. Brings receptive female into a mating situation

TABLE 10

REACTIONS OF AN ISOLATED MALE ANOLIAN TO INTRODUCED NONRESIDENTS
(Weight [Jan. 5], 4.2 Gm.; Average Length, 5.5 Cm.)

Date	Introduced Anolian	Reaction of Resident	Reaction Time	Response of Introduced Anolian
Nov. 29.....	♀	Antagonism	1'50"	Subordinate bob
Dec. 1.....	♀	No reaction	.....	
Dec. 3.....	♂	Antagonism	0 4	
Dec. 6.....	♂	Antagonism	1 0	
Dec. 8.....	♀	Antagonism	0 11	Subordinate bob
Dec. 10.....	♂	Antagonism	4 25	
Dec. 13.....	♀	Antagonism	0 25	
Dec. 17.....	♀	Antagonism	0 55	Subordinate bob
Dec. 18.....	♀	No reaction	.....	
Dec. 21.....	♂	Antagonism	1 5	
Jan. 5.....	♀	Courtship	.....	Subordinate bob
Jan. 7.....	♀	Antagonism	3 10	
Jan. 10.....	♀	Antagonism	8 20	
Jan. 17.....	♀	Antagonism	0 55	
Jan. 19.....	♀	Courtship	.....	Subordinate bob
Jan. 20.....	♀	Antagonism Courtship	0 4 2 40	Subordinate bob
Jan. 24.....	♂	Antagonism	0 30	
Jan. 26.....	♀	Antagonism Courtship	0 20 1 55	Subordinate bob
Jan. 28.....	♀	Antagonism	0 25	Subordinate bob
Feb. 7.....	♀	Courtship	0 35	
Feb. 9.....	♂	Antagonism mild	1 55	
Feb. 14.....	♀	Courtship	1 30	Subordinate bob
Feb. 16.....	♀	Courtship	1 35	Subordinate bob
Feb. 18.....	♀	Courtship	0 20	
Feb. 21.....	♀	Courtship	0 20	Subordinate bob

reactions to both males and females were secured until January, when an occasional alternate courtship of a female could be elicited. However, in February, females were consistently courted, while one male brought forth a mild fight reaction. Evidently, the young male was coming into rut and his responses reflected his changing physiological state.

SEX RECOGNITION

Noble and Bradley (1933) suggest a mechanism of sex recognition based on the presence of a dewlap in males and its absence in females:

> The male, after challenging the newcomer with an extension of his dewlap and receiving no answering challenge would swing into his courtship pattern. . . . . Attacked males call forth by a flourish of the dewlap the fight reaction of a male intent on mating. If the courting behavior is not checked in this manner, the attacking male continues until he has actually copulated with the male.

In the present study we have found that the extension of the dewlap is not the initial reaction of a dominant male toward a newcomer, nor does an attacked male immediately flourish his dewlap; and, finally, the attacker does not change to courtship when a large rival male fails to respond. The following quotations from early notes may serve to clarify these points:

> 1. February 20, 1936.—The newcomer, Male 9, slowly ascended to the upper part of the cage. Male 1 was resting there on top of the leaves; and, when Male 9 came into his line of vision, he assumed the following attitude: (1) throat swollen, (2) sides flattened, (3) dorsal ridge prominent—a formidable appearance. Male 9 seemed to realize the challenge and evidently was intimidated, because he edged sideways very slowly, moving away about 20 inches; Male 1 slowly relaxed his position.
>
> 2. February 26, 1936.—Male 13 moved up the screen and encountered Male 3, both edged sideways and parallel to each other about 8 inches apart, sides flattened, crest raised, throat swollen, no bobbing. Each seemed equally impressed by the other's display, maintaining the characteristic tense fight attitude. After 10 minutes of posturing, sidewise movements and head bobbing, Male 3 began determined movements toward 13, who gave ground and ran below. (Neither male displayed the dewlap as a preliminary challenge; and, in fact, it was not shown throughout the fight.)
>
> 3. April 10, 1936.—A fight began between Males 5 and 13. Male 1 soon took part. The large lizards were lined up parallel to each other, and Male 5 alternated in fighting Males 13 and 1, since he was in the middle. Male 17, also parallel to the fighting trio, showed the fight reaction but was disregarded. None of the four fighting males expanded his dewlap.

Evans (1935) assumed from his observations in small cages not only that males of *A. carolinensis* can "distinguish males from females but that dominating males can distinguish a strange male newly introduced into the cage from other males when none are in the fighting attitude." Yet he says in the same paper that "the actions of the courting male were always the same regardless of the sex of his mate." Evans (1938*b*) later concluded that a challenged female reveals her sex by bobbing in the female "courtship" manner. Since young males and even large subordinate males give this same head-nod response but are nevertheless recognized as males, its import in connection with sex discrimination required experimental verification.

The adults of *Anolis* show a distinct sexual dimorphism. The full-grown male is much larger than the female (Table 13). The dewlap of the latter is rudimentary, and she possesses no erectile dorsal and nuchal crest tissue. There are differences in the shape of the head which enable an experienced observer to distinguish the females in a group from young males of the same size. The male's head is broader and heavily muscled, while the female has more slender outlines. There is also a sexual difference in

the line of the throat; in the male the larger dewlap, with its extensor cartilage, causes a sagging, compared with the cleaner throat lines of the female. Such differences in appearance may possibly be used as cues or may be combined with behavioral signals to achieve sex recognition.

Between April 27 and June 24, 1937, we undertook to explore the whole matter experimentally. In the large cages (Table 1) we had observed each of the dominant males to court the females and attack resident suppressed males without confusion. Our experiments were based upon the introduction of various individuals into the cages in a prominent position and the recording of the responses of the dominant males to them, and vice versa. No attempts were made to test the females in this manner. We have at various times introduced large males into cages of females, and the latter usually responded with the subordinate gesture. Small males, instead, were often fought by the dominant females. In groups, females seldom nod to each other unless one is strongly dominant. The head nod is a reaction to size or aggression.

The criteria of recognition, i.e., evidence that the male lizard made a discrimination, were the differences between the behavior patterns: (1) characteristic head bobbing, (2) fight attitude not assumed in courtship, and (3) differences in approach (cf. Figs. 3 and 6). A basic assumption was made that a test male was recognized as such when he was fought with and a female when she was courted. Courtship of a male was considered as failure to recognize and not as proof of an inherent homosexuality.

When test animals were introduced into a cage, the alert dominant male usually approached immediately and showed a distinctive reaction; but, if he did not, the test lizard tended to conceal itself. Therefore, we found it useful in many cases to tie a short string to the latter's hind leg and anchor this to the screen of the cage with a bent nail. After several tugs, the lizard remained in place; and, when approached by the dominant male, its reactions seemed normal in every respect.

A total of 223 trials was made in a variety of experiments, many of which were not repeated sufficiently often to permit statistical evaluation. They are reported because they allow comparisons with situations that were more fully analyzed.

The initial reaction to females newly introduced into a cage was found to be in the nature of a challenge—i.e., the fight posture and dewlap display. Five males, each dominant in a different cage, reacted to a total of 73 normal, newcomer females; 64 elicited an initial fight reaction; and 9 females were immediately courted (Table 11). Resident females were "recognized" on sight. Of 17 resident females introduced within a few minutes under identical conditions as the newcomers, 14 were immediately courted. In addition to these cases, we have hundreds of recorded courtships of resident females and few, if any, cases of a dominant male initially showing fight toward a resident female.

Newcomer small males of approximately female size were initially responded to in the same way as the newcomer females; only 2 of 32 small males were initially courted. Large males of dominance size were invariably fought with.

Thus the sex of a small newcomer lizard is not "recognized" immediately. Overt signs of discrimination are observed later in the initial response, or, when cut short by flight, in subsequent responses. Thirty of the 64 initial fight

TABLE 11

RESPONSES OF 5 DOMINANT MALE ANOLIANS

	Responses of Introduced Anolians	Total No. of Individuals	1. Challenge 2. Fight	1. Challenge 2. Court	1. Challenge 2. Court 3. Fight	1. Challenge 2. Indifference	1. Courtship only	1. Court 2. Fight
	To Pegged Individuals							
Stranger ♀.....	No response	5	1	1	0	2	1	0
	Subordinate nod	35	4	20	0	9	2	0
	Other	1	1	0	0	0	0	0
Totals.....	.....	41	6	21	0	11	3	0
Resident ♀.....	Subordinate nod	4	0	2	0	1	1	0
	Other	1	0	1	0	0	0	0
Totals.....	.....	5	0	3	0	1	1	0
Small ♂.....	No response	6	2	2	2	0	0	0
	Subordinate nod	4	4	0	0	0	0	0
	Swelling of throat	4	4	0	0	0	0	0
	Subordinate nod and edge or more of fan	9	9	0	0	0	0	0
Totals.....	.....	23	19	2	2	0	0	0
	To Free Individuals							
Stranger ♀.....	No response	13	8	0	0	3	2	0
	Subordinate nod	19	4	9	0	2	4	0
Totals.....	.....	32	12	9	0	5	6	0
Resident ♀.....	No response	5	0	0	0	0	5	0
	Subordinate nod	7	0	0	0	0	7	0
Totals.....	.....	12	0	0	0	0	12	0
Small ♂.....	No response	3	0	2	0	0	1	0
	Subordinate nod	4	3	0	1	0	0	0
	Subordinate nod and edge or more of fan	2	1	0	0	0	0	1
Totals.....	.....	9	4	2	1	0	1	1
Large ♂.....	No response	3	3	0	0	0	0	0
	Throat swollen	1	1	0	0	0	0	0
Totals.....	.....	4	4	0	0	0	0	0

reactions to newcomer females ended in a switch to courtship; in 18 other cases the dominant male continued to fight or else the female ran out of sight, and 16 cases ended in indifference. In direct contrast, 23 of 30 challenge responses to newcomer small males continued as fight until the small male was either removed or ran away.

At some point in the 30 initial responses toward newcomer females which began with a hostile challenge, the dominant male changed to a response indicative of recognition of sex. Reading through the notes on these cases, we repeatedly find a sequence such as the following:

> Stranger female introduced, normal, not pegged. Dominant male sees her on screen from the opposite end of the cage; he is in full fight attitude, *shows fan repeatedly.*[4] The female doesn't move. He continues this fight challenge for several minutes; the female moves a little toward him without nodding. He approaches slowly, still in fight; the female also moves toward him, stops and nods. He releases the tense pose, comes closer, bobs and courts.

The female replied to the male's challenge with the head nod, which then appeared to elicit his courtship. However, there is another possible interpretation, namely, that the male exerts his dominance over all strangers and will withhold his courtship of a new female, although he may recognize her as such, until she shows subordination and until he is sexually excited.

A series of experiments were performed to test the role of response in sex discrimination. The eyelids of test lizards were sealed with collodion, so that they no longer responded to the dominant male's challenge. In 22 such trials the reacting male indicated an ability to recognize sex, in the absence of response, as shown by the fact that the 4 lizards which he courted were females (Table 12), and 2 of these were blinded to eliminate response. Six trials with large blinded males provided interesting results. In 4 cases they were attacked very ferociously (Table 12). For example, the following may be cited:

> May 28, 1937.—Introduced a large male, blinded and with collodion on the throat to prevent extension of the fan. The dominant male remained motionless for a moment on his lookout point, then moved onto the screen, fell off accidentally, moved up again, advancing toward the blinded male. He moved stiffly around his opponent, stalked him, tail twitching, and body very tense. He went through every fight maneuver, time and again bringing his open jaws in front of the blinded male's head and bending sideways, as if sparring. The fifth time he bit the male, causing him to move forward about 6 inches. He followed, circled around, stopped and nodded stiffly, circled, and made as if to spar with open jaws, then bit the male again. The latter ran; and the aggressor followed, biting at him until he fell to the ground.

In November, 1936, we introduced a large series of males of varying sizes into Cage H, where several large, pituitary-implanted males were active. Table 13 presents the results of these trials. It may be seen that, as the size of the introduced male departed more and more from that of the typical female, the frequency of fight reaction rose until a certain size elicited only an attack. Another equally large series of tests with a different set of males gave almost the identical picture. Only those cases were tabulated where the newcomers in turn showed no hostility; this was usually the case.

Size plays an important role in *Anolis* behavior. A prominent part of the fight pattern is increase in bluffing size. The dominant males are usually among the heaviest and largest (p. 411), and a conditioned correlation between size and

[4] Note that, while the dewlap is exhibited in threats toward females, small males, and subordinates, it is infrequently used in encounters between well-matched males.

TABLE 12

RESPONSES OF 5 DOMINANT MALE ANOLIANS TO EXPERIMENTALLY TREATED LIZARDS

Experimental Treatment	Individual	No. of Individuals	1. Challenge 2. Fight	1. Challenge 2. Court	1. Challenge 2. Indifference	1. Courtship only
Blinded (total absence of response)	Stranger ♀	8	2	2*	4	0
	Stranger small ♂	8	3	0	5	0
	Stranger large ♂	6	4	0	2	0
Artificial fan attached	Stranger ♀	6	5	0	1	0
	Resident ♀	8	5	0	2	1*
Throat swollen and painted	Stranger ♀	3	3	0	0	0
	Resident ♀	2	1	0	1	0
Throat swollen with saline	Stranger ♀	4	2	1*	1	0

* Only individuals courted are females.

recognition of maleness could easily arise. However, size and response are normally interrelated factors, since the larger the male, the stronger his reaction to a hostile challenge.

Two kinds of male responses were observed: (1) some part of the fight reaction and (2) the head nod. Whenever the test male showed any sign of fight, the dominant male was further activated and continued to attack until the male was out of sight. The gorged throat, dewlap display, or even the edge of the dewlap served to intensify his antagonism.

As a test of the importance of fight response in recognition, artificial dewlaps of crimson-colored paper were fastened to the throats of a series of 14 females, which were then introduced into the test cages (Table 12). Four other females received injections of saline under the throat skin to simulate the gorged throat of the fight pattern, and 5 more females had the throats both swollen and painted crimson. The males reacted very strongly to most of these females, even to residents which had just been courted. They were fought with in the same manner as small males giving a fight response. Females with throat injected and painted red were attacked as viciously as those females which were equipped with an artificial fan.

TABLE 13

EFFECT OF SIZE ON SEX RECOGNITION BY A SINGLE RESIDENT MALE ANOLIAN (1.2×1.9×6.0 CM.), IMPLANTED IN NOVEMBER WITH *Rana pipiens* ANTERIOR PITUITARY GLANDS

Male No.	Width of Skull (across Eyes)	Length of Skull (Ear to Tip of Snout)	Body Length (Tip of Snout to Anus)	Responses of the Resident Male
1	0.90 cm.	1.50 cm.	5.0 cm.	Fought, courted
2	0.90	1.50	5.0	Courted
3	0.90	1.50	5.0	Mated
4	0.90	1.50	5.0	Courted
♀	0.90	1.50	5.1	Courted
5	0.95	1.60	5.2	Fought, courted
6	0.95	1.65	5.4	Courted
7	1.00	1.65	5.4	Courted
8	1.00	1.60	5.5	Courted
9	0.95	1.70	5.8	Fought, courted
10	1.00	1.70	5.8	Fought
11	1.00	1.70	5.6	Fought, courted
12	1.05	1.70	5.5	Fought
13	1.05	1.70	5.8	Fought, mated
14	1.05	1.80	6.0	Fought
15	1.10	1.80	6.0	Fought
16	1.20	1.90	6.0	Fought
17	1.20	1.90	6.0	Fought
18	1.20	2.10	6.5	Fought

A number of other experiments were performed to test the relative importance of the factors of size and response. Large and small males were pegged together in the cage of a territory-holding male; in some instances both were normal, while in others one member of the pair was blinded to prevent response. The results indicate that, where an antagonistic reaction to the challenge was given, the dominant male directed his attention to the responder. In 5 cases the two were a large male and small male side by side; in 3 of these instances the smaller male bobbed in a subordinate manner but showed the edge or more of his fan—this turned the attack toward him. However, a large male giving a response was preferentially fought with even though the companion small male gave a stronger response. The following from our notes illustrates this point:

> June 24, 1937.—Introduced a large and a small male, pegged. The dominant male was on the other side of the screen; he bobbed and showed fan, came running over and attacked, moved around the small male who was in front and who bobbed and showed fan and challenged the large male, who merely had the throat swollen. The dominant male was now in full fight, bobbed, but did not show the fan. He continued to fight the large male until the latter was taken out, and then turned on the small male.

In another similar case, both the large and the small test males were quiet and the dominant male attacked the larger; this indicates that, in the absence of other responses, size itself is irritating to the male on territory, who centers attack upon the large individual. Even a slight cowering movement comes under the heading of response. Also, when two blinded animals were introduced together, the male challenged repeatedly at the slightest movement of either (3 cases). If only one of the pair was blinded so that it did not respond, this one was neglected; in the 1 observed case where the nonblinded member was a female, she nodded to the male and he courted, but in 2 other cases the nonblinded member was a small male, which likewise nodded but was nevertheless fought with. Hence recognition appeared to be independent of the type of response but rested rather on some sex dimorphism, the basis of which was not revealed by several types of preliminary experiments. In order to test this last important finding, 11 trials were run in each of which a male and female of the same size were introduced, pegged to the screen of the cage, side by side, about 6 inches apart. It was possible to determine which one the male was reacting to because he endeavored to hold his body parallel with his opponent at a distance of about 3 inches. In 5 cases the female was courted and the small male fought with, although they both nodded in subordinate fashion. The dominant male showed hostility toward both in 5 other instances, and in the remaining case fought the male and was indifferent to the female.

The finding that the small male's subordinate nod is insufficient to turn his challenger from fight to courtship casts doubt upon the significance of this response and raises the question of the manner in which the maleness of the small male is recognized. Although it is evident from our findings that a newcomer female usually must show subordination to the male before she is courted, the further deduction of Evans (1938*b*) that the head nod in some way reveals her sex to the male is not supported by our results. The most plausible explanation seems to be that sex dimorphism is not restricted to differences between the adults and that the small male differs sufficiently from a female of the same size that the dominant

male can become conditioned to this difference. There is implied a sensitivity of perception which the conservative observer hesitates to admit without more conclusive evidence.

In the field the small males would undoubtedly be more inclined to fight back than they are after handling and introduction into an unfamiliar setting. If males are distinguished mainly by their size and hostile response, then the smallness of the female and her failure to show fight or display the dewlap are factors in her recognition. However, there is evidence that sex recognition can operate on the basis of some sex dimorphism as yet undiscovered.

Functions of Color Display

IN THE FIGHT PATTERN

When accompanied by tense body posture and the deliberate bob of the fight pattern, the flourish of the dewlap is a challenge or threat which frequently is aimed at a male some distance away. Dominant males on adjacent territories threaten each other from vantage points, and the color of the dewlap may serve to advertise their presence to would-be intruder males. At closer range and in the course of a vigorous fight, the fan has a decidedly secondary role. It is usually not extended even when there are repeated bobbing motions. The fan is most often shown when the opponent is inferior, either a smaller or a subjugated male.

IN SEX RECOGNITION

A male defending his territory challenges all newcomers with the fight pattern and a show of color as well. In the preceding sections it was indicated that, while males of adult size are recognized in the absence of any response, the smaller males often betray their sex by fighting or partial dewlap extension. Therefore, color display may have a minor role in the recognition mechanism.

IN COURTSHIP

The courtship pattern almost always includes a flourish of the gular fan (Fig. 6). Since distinctive courtship movements are performed at the same time, it is difficult to evaluate separately the female's reaction to color. It appears that the entire pattern serves as a screening device whereby anoestrous females and immature individuals of both sexes are avoided as mating objects, since only receptive females stand their ground. This is merely another way of reporting the actual events.

Still another possible function of the entire courtship, including the color, is the gradual stimulation of the female toward oestrus. The history of each individual female reveals all stages of oestrous behavior leading to full receptivity. There is no evidence at hand regarding the role of the male's courtship in this progressive development.

IN DARWINIAN SEXUAL SELECTION

The simple hypothesis proffered by Darwin to explain the evolution of male sexual display characters, namely, that the female chooses as her mate the male which possesses the best-developed or showiest adornment, has received direct support only in rare instances. The most notable recent analysis is that of Noble and Curtis (1939) in the jewel fish (*Hemichromis bimaculatus*). *Anolis* offers still another especially favorable test material for this theory. A conspicuous secondary sex character is tucked away under the chin of the male, to be exhibited for the benefit of the female of the species whenever she is within visual distance. As Morgan (1919) suggested, "It is obvious that if a more brightly

colored male has a better chance of advertising himself to the female, all the essential requirements of Darwin's theory are fulfilled."

Therefore, we have attempted to test experimentally whether the female is attracted toward a male which shows color in preference to a rival artificially prevented from doing so. Such a test requires a special set of conditions peculiar to the species. There must be two adjacent but clearly separate territories with similar physical conditions, light, and food. Each needs to be defended by a similar male, one of which can then be prevented from showing color. Also, other residents of these areas must somehow be equivalent. In addition, the test females which are to make the "choice" have to be fully receptive, since anoestrous females are observed to run from courtship.

Early trials with this kind of setting were performed in large cages which two dominant males had divided into equal territories (see p. 413). In one trial, 10 females were closely watched for 15 observation days to discover where they usually stayed in the cage, and then one male's dewlap was prevented from opening by the application of collodion over a thin layer of cotton. Despite the fact that this male also suffered an injury to his back legs which hindered his movements, no significant changes in the distribution of the females were observed. A second trial consisted of the introduction of 10 new females into another cage in place of the old residents. They distributed themselves evenly between the two territories in this cage, and no effect of the closure of one male's fan could be detected. The male with suppressed dewlap courted and mated as successfully as the normal male.

Since caged lizards are under atypical conditions which may interfere in an experiment of this kind, all subsequent tests were made in the more natural environment of the entire greenhouse room. At intervals during a period from March 2, 1938, to July 19, 1938, various territory-holding males had their dewlaps either colored green with oil paint or suppressed with collodion, and their success in attracting and mating with females was tested against that of adjacent, normal males. Eleven such trials were run under a variety of conditions: in some the test females were established residents; in others new females were released midway between the two test territories; and, finally, in a few instances new males were released while the females were kept constant. In all these trials the male prevented from showing color was never at a disadvantage with regard to reproductive success. No evidence could be gathered in favor of an attraction-value of the red-colored dewlap.

Between August 10, 1938, and November 9, 1938, 5 additional trials of this kind of experiment were performed. The breeding season was already over, and the males and test females had to be implanted with *R. pipiens* anterior pituitaries to insure sexual activity. All these tests resulted negatively with regard to attraction to normal dewlap color. In the first 4 cases, a total of 17 implanted females were released midway between the two test areas. Of these, 8 settled with the male having a green-colored dewlap, 4 with the normal male, and 5 remained in some neutral place.

The fifth trial is of greater interest since it permits the separation of two distinct factors. Two pituitary-implanted males were encouraged to establish themselves on adjacent territories in the experimental area. All other lizards were removed; and 5 pituitary-implanted females, together with 5 nonimplanted

controls, were released equidistant between the two males. The males were caged at the end of each observation period and again replaced the following day; this procedure did not seem to interfere with their activity in any way. As soon as they were released, they would return to a favored spot and resume courtship display.

It was noted in preliminary tests that one male was somewhat more sexually active than the other. The dewlap of the more active male was painted green.

The test females in these experiments were all released within a fairly short distance of the males, never more than 3 feet away. There remained the possibility that color, while ineffective at short range, might function at a greater distance, in a manner similar to the distance threat. A new procedure was therefore devised (Fig. 16); two very similar territories about 13 feet apart were connected by 12-foot bridges to a central platform. A release box on this platform was a total visual distance of 13 feet

TABLE 14*

THE ROLES OF ACTIVITY AND COLOR IN ATTRACTING FEMALE ANOLIANS AT SHORT DISTANCE

Date	Green-Dewlap Male				Red-Dewlap Male			
	Activity		Females in Territory		Activity		Females in Territory	
	Courtship	Mating	Implants	Normal Control	Courtship	Mating	Implants	Normal Control
11/3/38.....	8	0	B, D	F	4	0	C	J, K
11/4/38.....	13	1	B,† D, E	F, I	7	0	C	G, J
11/5/38.....	12	1	A, B, D, E†	I	13	0	A?, C, E	F, G, J
11/6/38.....	5	0	A, B, D, E	I	4	0	C	F, G
11/7/38.....	4	0	B, D, E	.....	5	0	.....	F, G
Totals...	42	2	B, D, E	I	33	0	C	F, G, J

* A, B, C, D, E = implants; F, G, I, J, K = normal controls. Observations were made at intervals and not throughout the entire day.

† Mating.

After the females were released, this difference persisted. Table 14 presents the distribution of the females at the end of each day of observation. It may be noted that the male which courted more frequently also mated twice and that he had at least 3 of the pituitary-implanted and presumably oestrous females as consistent residents with him, whereas the normal male with red dewlap had only 1 such female. The controls exactly reversed this situation. These results seem to indicate that, if any attraction can be demonstrated, it is toward the more active male rather than to the specific color per se.

from each territory. The observer sat directly behind the box, hidden by a screen.

Each territory contained an active male and a group of resident females. Since it was found, early in the experiment, that the right-hand side seemed more favored by released females, the male on this side had his dewlap pinned down with collodion. His courtship and activity were not interfered with to any appreciable extent.

The observer placed a female in the release box, which had an outer shutter in addition to a glass release door. Both were raised by an overhead pulley. The

shutter was elevated when the males were in good position; the female in the box then had a clear view of the outside through the front glass door. The observer attempted to release the female when both males were courting at the same time in similar conspicuous positions. She came out along a narrow incline leading to the edge of the platform and then could turn and cross either bridge. Although the months of April and May had heretofore been found to be the time of seasonal oestrus for *Anolis*, it was thought advisable to implant the females with frog pituitaries in order to work with a standard group. Proof of oestrus was provided when the test female came across one of the bridges and was mated by the resident male.

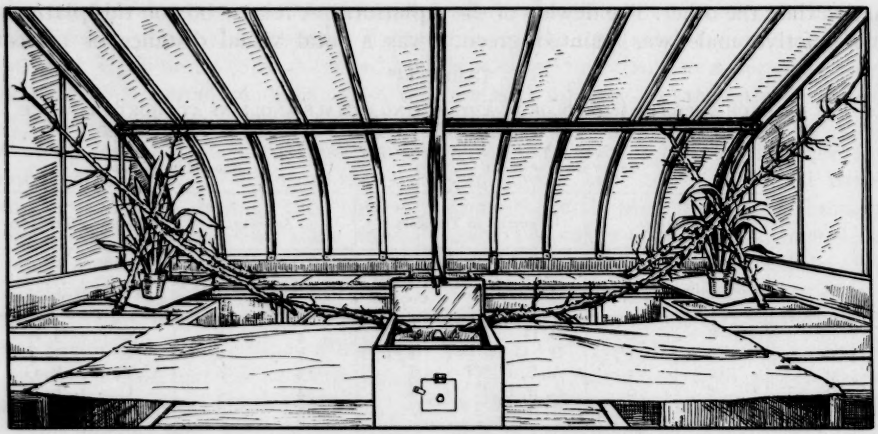


FIG. 16.—Sexual selection at a distance. The two closely similar territories were connected by 12-foot bridges to a central release box. A shutter and a glass door were raised or lowered by means of pulleys manipulated by the observer from a screen behind the box. The male in the right-hand territory was prevented from extending his dewlap by covering his throat with a bit of cotton and collodion, while the left-hand male was not interfered with. Females were released after 3–5 minutes of exposure to the courtship displays of these males, and they made their "choice" by crossing either bridge.

Unfortunately, there was no way of controlling the situation after the release door was opened. If one or both males completely disappeared, the shutter could be dropped; but the usual variation was in the number and intensity of courtships by the rival males. Also, a courtship chase could bring one male into prominence or conceal him.

Even when the males had displayed about equally well and the release came when both were courting at the same time, the female often did not start out at once, or the situation changed before she embarked on a definite path. For example, one male might stop while the other displayed continuously. In practice it was found that the experiment resolved itself into manipulating each trial as far as possible into the first of four situations at each release of a test female: (1) both males having courted about equally well, if possible together; (2) the color-displaying male courting, the other male having courted within the 3-5-minute period; (3) the nondisplaying male courting, the other male having courted within the previous 3-5-minute period; and (4) only the color-

displaying male having courted, the other male inactive or not visible.

The first of these situations seems to be most critical for added effect of color display; everything else is held approximately equal. The other three give the direction of unbalance at the time of release and subsequent reaction. If release comes when the color-displaying male is courting, the other male having courted first, then a preponderance of approaches toward color display indicates an attraction to courtship and perhaps to color. When the color-displaying male courts (within 3–5 minutes), then a preponderance of approaches toward color can be explained in two ways: (1) an initial attraction to the color-emphasized courtship which is "remembered" or is of sufficiently lasting impression that the nondisplaying male's courtship does not overcome it; or (2) at a distance of over 12 feet, the courtship of a nondisplaying male is not so clearly visible and does not make so strong an impression.

Attempts to control the situation presented to the test females were hampered by the fact that they could not be kept in an uncovered release box more than a certain standard time for all trials. If the critical point is relative visibility, subjection of a test female to unilateral display for too long a period might have a conditioning effect. It was found practical to raise the shutter when the situation looked promising and then to release within 3–5 minutes and as close to 5 minutes as possible. During that time the female almost always stayed at the front of the box, peering through the glass; and in several cases was observed to respond to the courtship of a male 13 feet away.

It might be considered that the improbability of ever getting two males to court with equal activity for equal periods of time weighed heavily against the experiment. The strength of this argument depends upon how delicate an effect of color we are looking for. A green male courting amid green leaves can hardly be detected by the human observer, but the flash of brilliant red is like a beacon light. However, the sensitivity of *A. carolinensis* to the dewlap color at the above distances has not been determined.

A total of 56 females was tested under these general conditions. Twenty-seven were implanted with *R. pipiens* anterior pituitaries; 9 were normals, tested during the breeding season; 13 were out-of-season controls; and 7 were spayed females. Table 15 gives the distribution of the results according to type of trial and female reaction (first runs only). The 9 normal females had been kept isolated from males since their arrival from the field and were probably close to oestrus at the time of the trials; they were not observed to mate. The implanted females were receptive, as shown by matings whenever they were allowed.

These data reveal a general trend toward the demonstration of an attraction value of color display at a distance. Perfect results were precluded, first, by the difficulty of controlling for color alone and, secondly, by its apparent weakness of stimulative effect. A male with dewlap suppressed can maintain territory and hold a group of females even in the face of a displaying male directly opposite, 13 feet away. Once resident in a male's territory, a female is usually not induced to desert by an adjacent rival's show of color.

In the preceding tests we found that oestrous females released at close range (3 feet or less) between two territories enter the territory not of the color-displaying but rather of the sexually more active male. Therefore, it would appear that color attracts mainly by virtue of

the degree to which it draws attention to the male's courtship movements. It is probable that all dewlap display, whether in courting or distance threat, has this secondary effect of revealing the presence of an adult male to unattached oestrous females; they may then migrate into his territory and become incorporated into a loose-knit mating group.

residents. Evans (1938*b*) summarized the fighting pattern of the male as

> a chain of eight responses which were overtly expressed in the following order: (*a*) Dewlap extension, (*b*) dorsal crest erection, (*c*) sidewise approach to the intruding male, (*d*) a lateral flattening of the body, (*e*) the biting response, (*f*) retreat response of the victim, (*g*) pursuit response of the victor, (*h*) dewlap response of the victor.

TABLE 15

SEXUAL SELECTION AT A DISTANCE

Introduced Females*	Both Males Courted		Color-displaying Male Courts, Then Nondisplaying Male		Nondisplaying Male Courts, Then Color-displaying Male		Only Color-displaying Male Courted		Totals	
	Fan	No Fan	Fan	No Fan	Fan	No Fan	Fan	No Fan	Fan	No Fan
Oestrous females:										
Implanted females	11	4	3	1	2	1	4	1	20	7
Normal females	6	0	1	1	1	0	0	0	8	1
Totals	17	4	4	2	3	1	4	1	28	8
Control females:										
Out-of-season females	1	4	1	1	0	6	0	0	2	11
Spayed females	1	1	0	0	0	1	1	3	2	5
Totals	2	5	1	1	0	7	1	3	4	16

* The response of the females consisted of movement toward the territory of one of the males, along a bridge leading from a central platform (Fig. 16).

Discussion

THE BEHAVIOR PATTERNS

The early naturalist literature contains occasional reference to the charming and colorful *Anolis "principalis"* (Lockwood, 1876; Monks, 1881; Shufeldt, 1883). Noble and Bradley (1933) first attempted a full description of its social behavior patterns. While the present study was in progress, there appeared a series of interesting papers by Evans (1935, 1936*a*, 1936*b*, 1938*b*), who observed the reactions of individuals isolated in small cages to introduced non-

These eight "reflexes" were said to be stereotyped and to appear invariably in the given sequence. We have observed that the initial fight reaction is always a gorging of the throat, followed almost immediately by lateral flattening of the body. Maintaining this tense posture, the male bobs stiffly, rising up on all four legs; he may or may not extend the dewlap at the second of his upward bobbing swings. The dewlap flourish is not essential to the fighting pattern, as shown by the fact that a male which has had this structure restrained by means of a collodion coating of the throat region is still

able to hold territory and win combats with normal rivals. Erection of the crest and a darkening of the skin just proximal to the eye are physiologically controlled responses; whether they are exhibited at all and how soon depends presumably upon the degree of emotional excitation. The active movements and maneuvers, such as sidewise advance, circling, and biting, are not rigidly stereotyped but are adapted to the reactions of the adversary.

Noble and Bradley (1933) considered the courtship of lizards to be simply a less extreme form of fight display; the sole distinction they made was that the mating male attempts to seize his victims by the nape of the neck. Evans (1938*b*) emphasized the role of the dewlap and "spasmodic body movements on stiffened forelegs." We find that courtship differs from fighting in two main respects: (1) a courting male does not assume the fight attitude of gorged throat and flattened sides; and (2) his bobbing motions are more rapid and become vibratory as he struts toward the female. Although they may follow each other in any sequence and in close succession, these two patterns are clearly separate as to form and probably motivation.

The function of the distinctive head-nod reflex has been disputed in the literature. Evans (1938*a*) reported some observations on two cages (dimensions not given), each of which contained 15 females and 2 or 3 males of the species *A. sagrei*. Whenever one of the males displayed the dewlap, the majority or all of the females gathered about him and nodded (p. 496, cases 22, 23). He concluded that "females in the presence of several males are attracted to the male which displays his dewlap most frequently thus showing sexual discrimination. Females have been observed to fight other females for possession of a male." This statement implies that the head nod is a sign of receptivity; in a second paper (Evans, 1938*b*) this gesture is considered to be the main element in the female's courtship pattern.

We have observed that receptive females may approach or remain near an active male and, as a result, may nod more frequently than the anoestrous females, which run or hide. However, defeated adult males, juveniles of both sexes, and spayed females have been observed to nod; this is obviously not always a sign of willingness to mate. There is likewise no direct support for the suggestions of Noble and Bradley (1933) and Walls (1942) that vertical head movements function as an aid to vision. The ease with which these lizards capture any moving or stationary prey without recourse to nodding and the fact that bobbing movements have become specialized with relation to each kind of behavior pattern indicate that these signals have a social, rather than an individual, value.

We have interpreted the head nod as a subordinate gesture. In many other animals, especially birds, there is a distinctive ceremonial gesture of submissiveness, which often takes the form of bowing or lowering the head. This response may free the subordinate from impending attack, or, as in the black-crowned night heron (Noble, Wurm, and Schmidt, 1938), it may be an essential prerequisite to pair formation. Precisely the same functions may be assigned to the head nod of the female anolian.

Motion attracts attention to the performer; therefore, as the result of the female's head-nod response, the male may court her more intensively. Without objective evidence it is unnecessary to make the result the purpose of this response.

In their discussion of the role of the female in courtship and copulation, Noble and Bradley were influenced by Wall's (1922) generalization that the female lizard is incapable of co-operating and must always be raped by the stronger males. They concluded that "a fundamental difference between the mating of lizards and that of birds is that the male lizards can mate with females at any time their own mating instincts develop." We have specifically disproved this statement by experimental tests (p. 401). Evans (1938*b*), on the other hand, stressed co-operation between the sexes in effecting copulation but described the female's receptive behavior as consisting of the head nod and slow retreat, "with perhaps an arching of her neck to facilitate his hold on it." Our observations suggest that in most matings the female does not retreat at all. Since this "stand" of the receptive female and the arch of the neck can be regularly stimulated in out-of-season or spayed females and even in males with either oestradiol or testosterone (Noble and Greenberg, 1941*a*, *b*) or with anterior pituitary implants in intact females, and inasmuch as spayed females never show this pattern, it may be concluded that oestrus in the lizard depends upon ovarian endocrine secretions. It would seem that copulation is possible only when ripe ova have developed to the point where the associated follicular or interstitial cells produce sufficient hormone to activate the female mating reflexes (Noble and Greenberg, 1941*b*). Introduction of sperm into the oviduct may be synchronized with maturation of the egg, so that fertilization occurs at the optimal time. Such mechanisms are, without doubt, widespread among the vertebrates but have not been extensively investigated; they are suggested, among others, by the studies of Noble and Curtis (1939) in the jewel fish, by Noble and Aronson (1942) in the leopard frog, by Skard (1936) with domestic chickens, and have actually been demonstrated by Young (1941) in a variety of mammals.

Sexual periodicity in female animals may sometimes be related to variations in aggressiveness. Evans (1938*b*) found that, after several months, isolated spayed female anolians responded to introduced nonresidents in hostile fashion much more quickly than did intact controls, and he concluded that the ovarian hormones inhibit fighting behavior. However, this theory is not supported by his study of the related Cuban species, *A. sagrei* (Evans, 1938*a*), and certainly not by our results—we find no evidence of either a negative or a positive correlation between oestrus and pugnacity.

A possible explanation of the excessive aggressiveness of some of Evans' spayed females can be suggested on the basis of our discovery (referred to above) of abnormal behavior, characterized by indiscriminate, aggressive mating attempts. Such animals, whether male or female, immature or adult, spayed or implanted with frog anterior pituitary glands, made biting attacks whenever in the presence of another lizard. Their reaction time was very brief, and there was a distinct lack of inhibition by any of the usually potent factors, such as strangeness of surroundings, large size of opponent, or unfavorable dominance relationships.

DOMINANCE AND WEIGHT

Some of the factors that influence success in fighting have been analyzed by Ginsburg and Allee (1942) in mice and by Collias (1943) in hens. These investigators found rather low positive correlations between dominance and weight. Evans (1936*a*) attempted to work out a hierarchy based on weight

among a group of 19 males of the species *A. carolinensis*. They were all placed in a small cage; and, as soon as one became tyrant, he was weighed and transferred to a second cage, where he was subsequently matched against the next male or males to crop out as dominant in the original group. This procedure was continued until a majority of the lizards had passed through 6 successive cages. Evans found that 90 of the 98 observed encounters were won by males which had already been resident in that particular cage for at least 24 hours before the loser was introduced. The effects of these two factors, weight and residence, were not adequately separated in the final analysis. Evans concluded that he had demonstrated "for the first time in any reptile form a social hierarchy which differs in many respects from that of fowls."

The terms "hierarchy" and "pecking order" have found wide use without exact delineation of their meanings. Many previous observers have noted the dominance of one male lizard over a group. It seems necessary to examine in what sense such relations constitute a hierarchical arrangement.

The term "hierarchy" is applicable to any social rank-order established through direct combat, threat, or passive submission (W. C. Allee, personal communication). Thus, two lizards may compose a hierarchy of two ranks, dominant and subordinate, while the tyrant and his subjugated group, providing the latter are all socially equal, are also at the two-rank level. In some species more complex relations are evolved, as, for example, in chickens, which usually form long pecking orders of many successive levels of dominance. In Evans' experiment no more than two ranks were ever involved, and his observations do not differ significantly from those of Noble and Bradley (1933) in this respect, except for interpretation.

The portion of Evans' data which bears directly upon the question of the relation between dominance and weight consists of the order in which his 19 animals became dominant in the original group (1936*a*, Table 2, p. 94). Each was weighed at the time he became tyrant; but, since the others were not weighed at the same time, a direct comparison with our similar experiment (Table 6) is difficult. Inasmuch as Evans' Male A was atypical, having been kept in the laboratory a full year before the arrival of the others and Male N was abnormal (*ibid.*, p. 103), these two should be omitted from consideration. Males O, P, Q, R, and S never dominated in the first cage. This leaves 12 males; the average weight of the first 6 differs from that of the lower 6 by 0.85 gm. ($p = 0.07$). This result is suggestive and compares well with our findings (Table 6). It supports the conclusion that during the breeding season the heavier males tend to be dominant over lighter ones, whenever the factor of territory does not overbalance the scale.

This correlation did not obtain among groups of males tested toward the end of the breeding season in July and August. It seems likely that, in this species, weight is merely an indicator of a complex interplay of such factors as age, size, strength, health, male hormone output, previous success in fighting, etc. We have not studied the relation between age and size or weight in male anolians, but the available observations suggest that within limits the smaller and lighter males are younger and less mature sexually. If weight is any indication of maturity, then it is also to that extent an indicator of male hormone production during the breeding season.

The older lizards are, without doubt,

the more experienced; and their greater size has been favorable to upward conditioning in relation to aggressiveness. This is a potent factor, as demonstrated by the work of Ginsburg and Allee (1942) with mice, which could be raised or lowered in dominance ranking at will by conditioning with an appropriate series of opponents. However, there seems little reason to suppose that equal increments in age have proportional effects on aggressiveness; nor is there any way at present of measuring quantitatively the effect of any given social situation on subsequent behavior, let alone the cumulative effects of very diverse situations presented in succession as in nature.

TERRITORIAL HIERARCHY

In the American chameleon the social group is integrated exclusively with relation to mating. When not breeding, individuals are generally isolated; and the males defend only their limited retreats, less strongly against females than against other males. Toward spring the lizards begin to range more widely, and they are aggressive within large areas. We have observed that at this time intense rivalry for territory may result in the formation of temporary three-rank hierarchies, sometimes of several weeks' duration. Eventually, either mutual toleration or the elimination of the second-ranking male terminates this unstable organization.

An apparently similar observation has been reported by Fitch (1941) in the western fence lizard. A resident male attacked and routed several newly released lizards but retreated from a second large resident because "it recognized the dominance of the larger male in its territory but would not tolerate other intrusions." However, Fitch points out that "fence lizard territories are not sharply defined or mutually recognized by individuals—seemingly each male defends an indefinite area which centers around his favorite lookout or basking point."

Many birds are strongly territorial in habit, and this has usually been found to preclude rigid social hierarchies (Collias, 1944). However, a striking example of a territorial hierarchy may be seen in the male sage grouse (Scott, 1942). At each mating ground the males are organized into four levels of dominance, with the major share of mating privileges falling to the "master" cock, followed by a partially suppressed challenger or "subcock," and finally a circle of apparently younger "guard" cocks, which aid the defense of the mating area against newcomers.

This phase of territorialism has not been widely studied. Nice (1943) states that "with a territorial bird the support from its own piece of land is so strong that it is undefeatable on its own territory." Whether there is a valid difference between lizards and birds in this respect needs to be tested in the field environment.

GROUP FORMATION

In polygamous animals the male forms separate bonds with each female of his harem. The variations in degree of integration of such groups have been summarized by Collias (1944). In *Anolis* the bonds are place-conditioned (the common territory) and are also, to an undetermined extent, the result of familiarity or recognition of individuals. Parental duties are not shared; and mating ties are of relatively brief duration, compared with those of many birds and mammals.

The pairing process combines sex recognition and selection mechanisms. Adult territory-holding males are large, and their dewlap display is visible at a

distance. Unpaired females tend to gravitate toward such conspicuous males. Thus, in the case of the females, recognition and selection may occur simultaneously. On the other hand, the male need only recognize a female as a mating object; he welcomes any female newcomer with special courtship vigor and excludes no female from his harem.

The mechanism of sex recognition by the male is more readily studied than that used by the female lizard. The former responds differentially, fighting with other males and courting the females. The problem then resolves into an analysis of the signals shown by males which arouse antagonism, as against the cues afforded in the appearance or behavior of the females. In one sense, the female's repertoire of responses to test lizards is more limited than that of the male. She may exhibit the fight pattern, the subordinate gesture, oestrus (neck bend), or flight; essentially, these represent either dominance or subordination. Since females may also be subservient to members of the same sex and, when treated with testosterone, may nevertheless take the female role in copulation with other females (Noble and Greenberg, 1941*a*), it is possible that they respond to dominance and not to sex per se. The neck-bend reflex, however, is specific to a mating situation, whether the courting individual is a male or a testosterone-treated female.

A dominant male challenges each newcomer with the fight attitude. Since the females immediately show subordination while the males tend to fight back, it has been assumed that this difference in response determines the male's subsequent behavior. Lorenz (1935) based his "lizard type" of sex recognition on Noble and Bradley's (1933) description of this mechanism. Tinbergen (1939) discusses a basically similar kind of discrimination test in species with poorly developed dimorphism. In the American chameleon there is evidence that effective cues for sex recognition are present even in the absence of distinctive behavior responses. This is easily accounted for by the pronounced sex dimorphism in this species. Size, shape of head, and throat lines are the characters most likely to function in this manner. A surprising result is the finding that the small male, which may nod subordinately in female fashion to a dominant male, is nevertheless attacked. This suggests that, although the female must show subordination by nodding, she either lacks certain male signals or possesses others which enable the male to identify her as a mating object. It is notable that, after the formation of a mating bond, resident females are seldom again challenged by the dominant male.

It has long been doubted whether sexual selection of any kind exists in lizards. For instance, Huxley (1938) considers the colors of male lizards to be threat characters, effective only at short range. This is not the case in *Anolis;* color display, whether in threat or in courtship, may well be an adaptation that provides conspicuousness at a distance. Exception must be taken to his general statement (*ibid.*, p. 30) concerning lizards

> in which no courtship-display exists, the female being either entirely passive in regard to coition, or else having to be subdued by force or by special soothing organs; and the male's bright colours are entirely directed against trespassers and rivals of the same sex.

This is a rather unfortunate misconception in the literature (cf. also Lorenz, 1935). *Anolis* provides, in fact, an excellent case of intrasexual selection.

It is difficult to place the lizards in Huxley's scheme of levels of reproductive organization (1938, p. 37, Table 2).

They would seem to fit best alongside the ruff and the blackcock, rather than where the author puts them, since territorial mating polygamy is achieved through combined combat and display. However, Huxley's classification lacks a measure of the degree of integration. It is based on the manner in which bonds are formed rather than on how well organized the groups become or their duration.

SUMMARY

The social behavior patterns of *A. carolinensis* were studied in the laboratory during the period of February 2, 1936, to December 18, 1940. Use was made of specially adapted experimental techniques as well as observation of groups under close-to-normal conditions.

The fighting, courtship, and mating patterns of the male; oestrous and aggressive behavior of the female; oviposition; the subordinate gesture; and two forms of sexual aberrations shown by both sexes—all are fully described.

During the breeding season the males defend restricted territories within which groups of females may become resident. In early stages of boundary disputes, temporary three-rank hierarchies are sometimes established among adjacent males; these are terminated by either mutual respect of a boundary line or the disappearance of the second-ranking male. The females also become organized in straight-line hierarchies of brief duration.

A greater correlation between dominance and weight was found among males during the breeding season than out of it. Since size and weight are directly correlated with age, and therefore also with sexual maturity and experience, it is suggested that the older, larger, and more experienced males tend to be dominant at that time.

The process of formation of mating groups involves apparently simple sex recognition and sexual-selection mechanisms. A territory-holding male challenges all newcomers and thereby usually elicits distinctive responses which aid his recognition of sex. However, in the absence of such responses, evidence was secured of an ability to use cues that were not detected in this series of experiments.

Oestrous females tend to approach males which make themselves conspicuous through dewlap display. This type of sexual selection is ineffective at short range. The color of the gular fan is not innately attractive but would rather appear to enhance the male's courtship movements so that they are more easily visible to receptive females.

LITERATURE CITED

ALLEE, W. C. 1931. Animal aggregations: a study in general sociology. Chicago: University of Chicago Press. Pp. 431.

———. 1938. The social life of animals. New York: W. W. Norton. Pp. 293.

———. 1942. Social dominance and subordination among vertebrates. A chapter in Levels of integration in biological and social systems, edited by R. Redfield. Lancaster, Pa.: Jacques Cattell Press.

———. 1943. Where angels fear to tread: a contribution from general sociology to human ethics. Science, **97**:517–25.

BARBOUR, T. 1926. Reptiles and amphibians: their habits and adaptations. Boston and New York: Houghton Mifflin. Pp. 125.

BEACH, F. A. 1942. Arousal, maintenance and manifestation of sexual excitement in male animals. Psychosomat. Med., **4**:173–98.

COLLIAS, N. E. 1943. Statistical analysis of factors which make for success in initial encounters between hens. Amer. Nat., **77**:519–38.

———. 1944. Aggressive behavior among vertebrate animals. Physiol. Zoöl., **17**:83–123.

CRAWFORD, M. E. 1939. The social psychology of the vertebrates. Psychol. Bull., **36**:407–45.

EVANS, L. T. 1935. Winter mating and fighting behavior of *Anolis carolinensis* as induced by pituitary injections. Copeia, No. 1, pp. 3–6.

———. 1936*a*. A study of a social hierarchy in the lizard, *Anolis carolinensis*. Jour. Genet. Psychol., **48**:88–111.

———. 1936*b*. Territorial behavior of normal and castrated females of *Anolis carolinensis*. *Ibid.*, **49**:49–60.

———. 1938*a*. Cuban field studies on territory of the lizard, *Anolis sagrei*. Jour. Comp. Psychol., **25**:97–125.

———. 1938*b*. Courtship behavior and sexual selection of *Anolis*. *Ibid.*, **26**:475–98.

FITCH, H. S. 1941. A field study of the growth and behavior of the fence lizard. Univ. Calif. Pub. Zoöl., **44**:151–72.

GELDERN, C. E. VON. 1919. Mechanisms in the production of the throat-fan in the Florida chameleon, *Anolis carolinensis*. Proc. Calif. Acad. Sci., **9**:313–29.

GINSBURG, B., and ALLEE, W. C. 1942. Some effects of conditioning on social dominance and subordination in inbred strains of mice. Physiol. Zoöl., **15**:485–505.

GREENBERG, B., and NOBLE, G. K. 1942. Dominance, social order and territory in the lizard. Anat. Rec., **84**:58 (abstract).

HUXLEY, J. S. 1938. The present standing of the theory of sexual selection. A chapter in Evolution: essays on aspects of evolutionary biology, edited by G. R. De Beer. Oxford: Clarendon Press.

KLEINHOLZ, L. H. 1938. Studies in reptilian colour changes. II. The pituitary and adrenal glands in the regulation of the melanophores of *Anolis carolinensis*. Jour. Exper. Biol., **15**:474–91.

LOCKWOOD, S. 1876. The Florida chameleon. Amer. Nat., **10**:4–16.

LORENZ, K. 1935. Der Kumpan in der Umwelt des Vogels. Jour. f. Ornith., **83**:137–213, 289–413.

MONKS, S. P. 1881. A partial biography of the green lizard. Amer. Nat., **15**:96–99.

MORGAN, T. H. 1919. The genetic and operative evidence relating to secondary sexual characters. Carnegie Inst. Wash., Pub. No. 285.

NICE, M. M. 1943. Studies in the life history of the song sparrow. II. The behavior of the song sparrow and other passerines. Trans. Linnaean Soc., New York, **6**:1–329.

NOBLE, G. K. 1939. The experimental animal from the naturalist's point of view. Amer. Nat., **73**: 113–26.

NOBLE, G. K., and ARONSON, L. R. 1942. The sexual behavior of Anura. I. The normal mating pattern of *Rana pipiens*. Bull. Amer. Mus. Nat. Hist., **80**:127–42.

NOBLE, G. K., and BRADLEY, H. T. 1933. The mating behavior of lizards; its bearing on the theory of sexual selection. Ann. N.Y. Acad. Sci., **35**: 25–100.

NOBLE, G. K., and CURTIS, B. 1939. The social behavior of the jewel fish, *Hemichromis bimaculatus*. Bull. Amer. Mus. Nat. Hist., **76**:1–76.

NOBLE, G. K., and GREENBERG, B. 1939. Social behavior and sexual selection of the Florida chameleon. Bull. Ecol. Soc. Amer., **20**:28 (abstract).

———. 1941*a*. Induction of female behavior in male *Anolis carolinensis* with testosterone propionate. Proc. Soc. Exper. Biol. and Med., **47**: 32–37.

———. 1941*b*. Effects of seasons, castration, and crystalline sex hormones on the urogenital system and sexual behavior of the lizard, *Anolis carolinensis*. I. The adult female. Jour. Exper. Zoöl., **88**:451–74.

NOBLE, G. K.; WURM, M.; and SCHMIDT, A. 1938. Social behavior of the black-crowned night heron. Auk, **55**:7–30.

PETIT, G. 1928. Sur le *Chalarodon madagascariensis* Peters. Bull. Soc. zool. de France, **53**:401–5.

RUGH, R. 1937. A quantitative analysis of the pituitary-ovulation relation in the frog (*Rana pipens*). Physiol. Zoöl., **10**:84–100.

SCOTT, J. W. 1942. Mating behavior of the sage grouse. Auk, **59**:477–98.

SHUFELDT, R. W. 1883. Observations on the habits of the American chameleon (*Anolis principalis*). Amer. Nat., **17**:919–26.

SKARD, A. G. 1936. Studies in the psychology of needs: observations and experiments on the sexual need in hens. Acta psychologica (Hague), **2**:173–232.

TINBERGEN, N. 1939. On the analysis of social organization among vertebrates, with special reference to birds. Amer. Midl. Nat., **21**:210–34.

WALL, F. 1922. Notes on some lizards, frogs, and human beings in the Nilgiri Hills. Jour. Bombay Nat. Hist. Soc. (Calcutta), **28**:493–99.

WALLS, G. L. 1942. The vertebrate eye and its adaptive radiation. Cranbrook Inst. Sci. Bull., No. 19.

YOUNG, W. C. 1941. Observations and experiments on mating behavior in female mammals. Quart. Rev. Biol., **16**:135–56, 311–35.

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